# Exposure to Phthalate Mixtures and Inner-City Pediatric Allergic Disease and Airway Inflammation

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**Abstract**

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Phthalate plasticizers are found in consumer products and home furnishing materials. Phthalate urinary metabolites are detected in nearly every sample in population-based studies indicating widespread exposure. Prior epidemiologic studies have associated vinyl flooring, a proxy for phthalate exposure, or house dust concentrations of phthalates with eczema and asthma in children. However, these studies lack adequate exposure measurements, consideration of the early life period, and prospective designs. In light of these gaps in the literature, we designed epidemiologic analyses to address our overarching hypothesis that early life exposure to a mixture of phthalates will have associations with adverse allergic and respiratory health outcomes in children. We tested this hypothesis in five self-contained manuscripts that characterize sources of exposure to phthalates in early life, demonstrate the application of new statistical methods for estimating effects of these highly correlated biomarkers, and test the association between early life exposure to phthalates and eczema and airway inflammation in children.

Participants were enrolled from the longitudinal birth cohort of the Columbia Center for Children's Environmental Health (CCCEH) in New York City. Phthalate metabolites were measured in prenatal and child urine samples at the Centers for Disease Control and Prevention. Questionnaires and visual inspections were combined with phthalate measurements from personal

and indoor air sampling and urinary metabolite concentrations to examine sources and patterns of phthalate exposure associated with personal care product use and flooring materials in the home.

The use of perfume and personal care products was associated with higher exposure to the metabolite of diethyl phthalate (DEP) but not di-*n*-butyl phthalate (DnBP). Vinyl flooring in the home was associated with higher indoor air and urinary metabolite concentrations for butylbenzyl phthalate (BBzP) but not di(2-ethylhexyl) phthalate (DEHP).

Because some phthalates share exposure sources and have multiple metabolites, the urinary biomarker concentrations can be highly correlated. Using a reanalysis of the association between prenatal phthalate metabolites and reduced gestational age, we demonstrate that simple Bayesian models can estimate effects for highly correlated exposure measures without the instability of conventional modeling approaches.

We found that prenatal concentrations of the metabolite of butylbenzyl phthalate were associated with the report of early-eczema but not atopy among children in the cohort. In a cross-sectional analysis, children's urinary concentrations of metabolites of diethyl phthalate and butylbenzyl phthalate were both associated with higher fractional exhaled nitric oxide, a marker of airway inflammation.

These findings suggest several important sources of exposure to phthalates and demonstrate new methods for highly correlated exposures that have not been widely applied in the environmental health sciences. The association of biomarkers of exposure to butylbenzyl phthalate and eczema extend the findings of previous studies. Our results include the first report of an association between phthalates and airway inflammation in children.

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## Preface

This dissertation consists of a statement of hypotheses and specific aims, a background, five self-contained studies, a brief conclusion, and next steps. Each of the self-contained studies is organized as a scientific manuscript with its own abstract, introduction, methods, results, discussion, and references. As all scientific studies of this type are collaborative, the coauthors for each of these studies are named at the beginning of the respective chapters.

The hypotheses and specific aims are the overarching questions that motivated this research. Each aim is addressed in one or more of the five self-contained studies. Specifically, the first two studies (Chapters 3 and 4) address the first specific aim of the dissertation: to identify sources and contributions to phthalate exposure in populations of concern, namely pregnant women and their children. These studies use biomarker and environmental exposure measurements along with data collected through surveys to study personal care products as potential sources of diethyl phthalate (DEP) and di-*n*-butyl phthalate (DnBP) during pregnancy and vinyl flooring in the residential environment as a potential source of butylbenzyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP) during childhood.

The third study (Chapter 5) addresses the second specific aim of the dissertation: to develop a statistical approach for estimating associations between multiple correlated biomarkers and a health outcome measured on a continuous scale. The manuscript compares two commonly used statistical methods for highly correlated biomarkers with two simple Bayesian models. Because these methods have not yet been widely applied in the epidemiologic literature, and have not previously used biomarkers as continuous exposure estimates nor assessed the relationship to a continuous outcome measure, our study began with a reanalysis of an already published epidemiologic association between DEHP metabolites and reduced gestational age. This was

deemed an appropriate starting point to compare modeling approaches, as it had already been thoroughly analyzed with conventional statistical approaches including a careful consideration of potential confounders. Furthermore, this reanalysis particularly involved associations with the DEHP metabolites which are the most highly correlated subset of the phthalate metabolites. As an appendix to this study we include code utilizing free software tools so that others could potentially apply these methods in other situations with multiple correlated exposures.

The fourth and fifth studies (Chapters 6 and 7) address the third specific aim: to examine associations between prenatal and children's exposure to phthalates and health outcomes. The fourth study focuses on the association between prenatal exposure to BBzP and allergy related outcomes in children. The hypothesis is based on results from prior research associating house dust concentrations of BBzP with child eczema. The fifth study examines the association of children's urinary metabolite concentrations for all four of the primary phthalates of interest (DEP, DnBP, BBzP and DEHP) and their association with concurrent fractional exhaled nitric oxide (FeNO), a marker of airway inflammation.

The background section of the dissertation provides a brief introduction to the topic areas and describes the connection between the five studies, their aims and approaches. However, detailed background specific to each of the self-contained studies is provided in the manuscripts themselves. Similarly, the methods sections are provided in the manuscripts. Finally, the overall conclusion seeks to summarize how these five studies address the specific aims of this dissertation, as well as discuss the relevance of the major findings and future directions for research in these areas. It augments, but does not repeat, the detailed discussion contained in each of the five manuscripts.

## Chapter 1: Statement of hypotheses

This dissertation is an epidemiologic investigation of the sources of exposure to phthalates during pregnancy and early childhood, methods for statistical analysis of correlated biomarkers of phthalate exposures, and associations between exposures to phthalates and allergic and respiratory health outcomes in children. Phthalates are plasticizers commonly used in household and consumer products to confer flexibility. They are also used as solvents and fixatives in fragrances and personal care products. Widespread use has resulted in ubiquitous exposure to multiple phthalates. Recent epidemiologic studies show cross-sectional associations between several phthalates in house dust and childhood asthma and allergic diseases. However, patterns of prenatal and childhood exposure to phthalates are not well understood and population-based studies that use biomarkers of exposure are needed to establish whether or not phthalate exposure during pregnancy and early-life plays a role in pediatric allergic and respiratory disease.

This epidemiologic study, conducted within an ongoing longitudinal birth cohort, fills these important research gaps. The research has four critical components: 1) the measurement of established biologic and environmental dosimeters of exposure to phthalates during pregnancy and early childhood; 2) the exploration of statistical methods for modeling effects of multiple correlated exposures; 3) the use of validated child outcome measures including proallergic immunoglobulin E (IgE) production, and fractional exhaled nitric oxide (FeNO) as a measure of airway inflammation; and 4) the use of a prospective birth cohort design to evaluate the impact of prenatal and early-life phthalate exposures on later childhood immunologic and respiratory outcomes. The following four phthalates will be evaluated: diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), butylbenzyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP). This research seeks to address a single unifying hypothesis: **Early life exposure to a mixture of**

**phthalates will have associations with adverse allergic and respiratory health outcomes in children.** We operationalized this hypothesis in three parts that dealt with exposure, statistical methods, and associations with health outcomes:

*Hypothesis 1:*

We predict that the use of personal care products and flooring materials in the home environment contribute to patterns of prenatal and childhood phthalate exposure. Specifically, we hypothesize that the use of perfume and other personal care products is associated with higher exposure to DEP and DnBP and that the presence of vinyl flooring materials that may contain phthalates is associated with higher exposure to BBzP but not DEHP.

*Specific Aim 1: sources of prenatal and childhood exposure*

We will characterize patterns of prenatal and early childhood exposure to phthalates in an urban cohort using questionnaire data on maternal use of personal care products and a checklist of materials in the home and examine associations with concentrations of phthalates measured in personal and indoor air samples, and the urinary metabolites measured in prenatal and child urine samples.

*Hypothesis 2:*

We hypothesize that simple Bayesian regression models can estimate associations between multiple correlated phthalate biomarker measures and a continuous health outcome and that these estimates will better reflect actual mixtures and be more stable than estimates from single biomarker or full linear regression models.

*Specific Aim 2: models for correlated biomarkers*

We will construct simple Bayesian regression models to reanalyze an association of prenatal urinary metabolites with reduced gestational age and compare methods utilizing overall or grouped shrinkage with models fit for single metabolites and a full linear regression model with many correlated phthalates as predictors.

*Hypothesis 3:*

We predict that higher prenatal exposure to butylbenzyl phthalate will increase risk of early childhood eczema and production of proallergic immunoglobulin E (IgE) antibodies by age 5. We also predict that exposure to the four phthalates will be associated with increased concurrent airway inflammation (FeNO) among cohort children.

*Specific Aim 3a: eczema and atopy.*

We will develop multivariable linear regression models to examine the association between prenatal urinary concentrations of mono-benzyl phthalate (MBzP) and questionnaire based maternal report of children's eczema as well as total serum IgE and allergen specific IgE measured at ages 2, 3, and 5 years.

*Specific Aim 3b: airway inflammation*

We will use regression models to examine the cross-sectional association of urinary phthalate metabolite concentrations with airway inflammation (FeNO) between ages 5-9.5. In addition, we will assess whether there is effect modification that varies these associations among children who are seroatopic or prone to wheeze.

## Chapter 2: Background

### *Sources of phthalates*

Phthalates are the diesters of 1,2-benzenedicarboxylic acid (phthalic acid) and are semi-volatile organic compounds. They are commonly used plasticizers added to polymer based materials and consumer products, including up to 40% of polyvinyl chloride plastics, to confer flexibility or durability (NTP-CERHR 2006). They are also used as solvents and fixatives in fragrances and personal care products (Api 2001; ATSDR 1995; Cosmetic Ingredient Review Expert Panel 2005; Hubinger and Havery 2006). Phthalates are found in vinyl flooring, adhesives, pharmaceutical coatings, medical devices, personal care products, children's toys, and as food contaminants among many other sources (Chou and Wright 2006; Schettler 2006). Although they are often referred to as a collective group, there are many phthalates in widespread commercial use with different predominant uses, physical and chemical characteristics, and toxicologic profiles for each. The four phthalates (DEP, DnBP, BBzP, DEHP) that are the main focus of this study are of particular interest based on previous epidemiologic and toxicologic studies discussed below. The chemical structures of these four phthalates are shown in Figure 1 (Frederiksen et al. 2007).

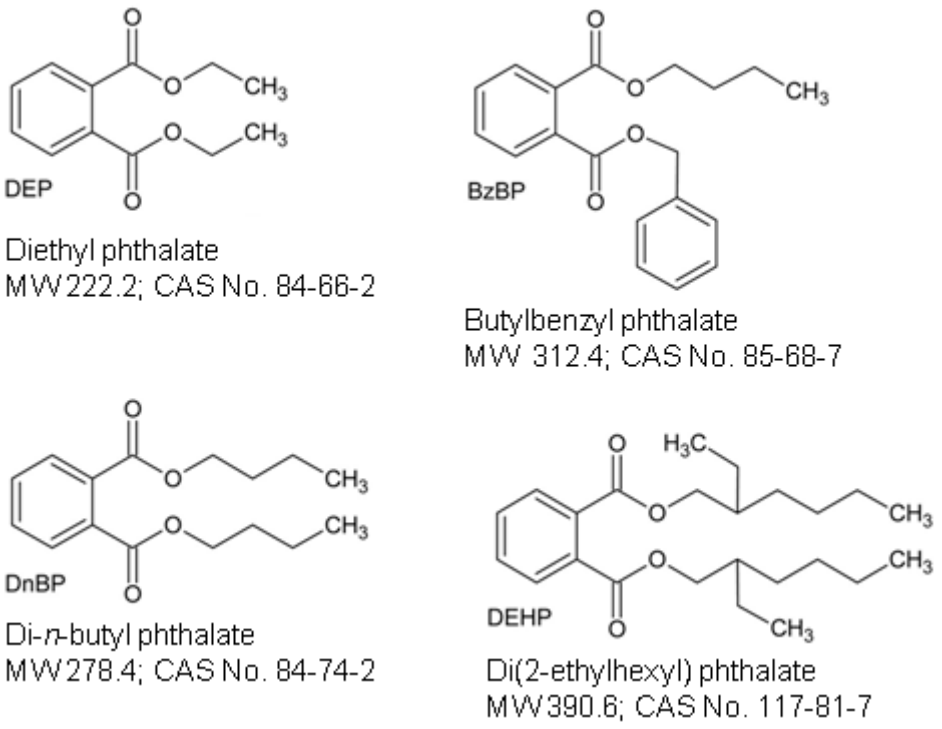


**Figure 1: Chemical structures of four phthalates of interest**

*Exposure and measurement*

These four phthalates of interest are high production volume chemicals. Population based studies, including nationally representative studies in the United States (NHANES), found evidence of nearly ubiquitous exposure via the measurement of metabolites in urine (Silva et al. 2004). In the Columbia Center for Children's Environmental Health (CCCEH) study of Mothers and Newborns we detect metabolites of the four phthalates in >99% of prenatal and child urine samples (Adibi et al. 2008; See Chapter 7). Phthalate metabolites are also detected in blood, and breast milk (Frederiksen et al. 2007), with evidence of transplacental exposure (Wittassek et al. 2009). The development of laboratory methods to analyze phthalate metabolites in biologic media has enabled a growing body of epidemiologic health studies that utilize these measures. Many of the current laboratory methods were first developed at the National Center for Environmental Health at the Centers for Disease Control and Prevention, the laboratory that analyzed phthalate

metabolite concentrations for these studies. These methods utilize an on-line solid-phase extraction coupled with high performance liquid chromatography and tandem mass spectrometry (Kato et al. 2005). Limits of detection for the various metabolites are less than one ng/ml. Because there is no esterase activity in urine samples to hydrolyze parent diester phthalates to the first stage monoester metabolites, the concentrations of urinary metabolites are not susceptible to contamination with phthalates that might occur through sample collection or handling (Hauser and Calafat 2005).

The measurement of phthalates in urine is a valuable biomarker for epidemiologic studies of health effects because it integrates exposure across multiple routes: ingestion, inhalation, and dermal absorption. This is an advantage in studies in which total uptake might be more important than an estimate of uptake by a single route, such as estimating prenatal exposure from biomarker concentrations in maternal urine. However, the inability to separate routes of exposure can be a limitation when investigating mechanisms of biologic effect particular to intake via a specific route. The contribution of each of the routes of exposure appears to vary between phthalates (Fromme et al. 2007; Wormuth et al. 2006), and urinary concentrations of different phthalate metabolites typically span orders of magnitude within a population (Silva et al. 2004). The variability in concentrations of phthalates in environmental media and biologic samples can arise from variation in the sources and concentrations, individual's behaviors, and differences in metabolism and excretion. Details on metabolism, half-lives, and the reliability of the biomarkers are discussed in the self-contained manuscripts.

Understanding the contribution of different sources of phthalate exposure is important for identifying groups with abnormally high exposures as well as designing interventions that could lower exposure. Prior studies looking at source contributions to phthalate exposures have provided limited insights but often are not able to account for the multiple routes of exposure or for the high

degree of variability across populations. Examples of experimental or interventional study designs have included topical application of a cream with a known concentration of several phthalates to monitor dermal uptake (Janjua et al. 2008), and measurement of urine concentration before and after extended fasting to evaluate dietary contribution (Wittassek et al. 2011). These designs provide powerful evidence through experimental manipulation but can be of limited generalizability as they are typically done with small sample sizes of adult volunteers. Exposure studies have also measured phthalate concentrations in dietary sources, consumer products and environmental media including dairy products (Castle et al. 1990), personal care products (Hubinger and Havery 2006), and indoor air and house dust (Rudel et al. 2003). Human exposures have been estimated by models that combine these source concentrations with time-activity data (Wormuth et al. 2006). Due to the high variability in exposures measured in population-based studies, observational studies testing associations with putative environmental sources have succeeded in identifying contributors to exposure or internal dose only when a particular source/behavior is the dominant contributor. For example, the urinary concentration of the metabolite mono-*n*-butyl phthalate was 49 times higher in the six NHANES participants reporting use of the polymer coated medication Mesalamine compared to the mean concentration among nonusers (Hernandez-Diaz et al. 2009).

Our data and others' indicate that exposure to lower molecular weight phthalates such as DEP is positively associated with use of personal care products in pregnant women (Berman et al. 2009; Just et al. 2010), and infants (Sathyanarayana et al. 2008b). Because of their lower molecular weight, these phthalates are also found at higher concentrations in indoor air compared with higher molecular weight phthalates such as DEHP and BBzP (Adibi et al. 2008; Rudel et al. 2003). Models suggest that exposure to the higher molecular weight phthalates, especially DEHP, is

primarily dietary in adults (including pregnant women) and school age children (Fromme et al. 2007; Koch et al. 2006; Wormuth et al. 2006). In addition, higher molecular weight phthalates used in consumer products, furnishings, and building materials accumulate in interior environments bound to particles in dust (Abb et al. 2009; Becker et al. 2004; Bornehag et al. 2005a; Fromme et al. 2004; Kolarik et al. 2008a; Langer et al. 2010; Rakkestad et al. 2007; Rudel et al. 2003; Rudel et al. 2010). The higher molecular weight phthalates, including BBzP and DEHP, have a low vapor phase but can be resuspended on dust particles, including respirable particles (Rakkestad et al. 2007) by indoor air circulation. Dust and air concentrations have been shown to be correlated (Oie et al. 1997; Rudel et al. 2003; Xu et al. 2009). The correlation among pregnant women in the CCCEH cohort between personal air phthalate diesters and maternal urinary metabolite concentrations for several phthalates indicates that inhalation may also be an important contribution to exposure to certain phthalates, particularly DEP and BBzP (Adibi et al. 2003; Adibi et al. 2008). Our results also indicate that perfume use among pregnant women in the CCCEH cohort may be a potentially large source of dermal exposure to DEP (Just et al. 2010). Infants and toddlers have additional exposures to phthalates via ingestion of dust and hand/toy to mouth activity (Sathyanarayana et al. 2008a; Sathyanarayana et al. 2008b; Wormuth et al. 2006).

*Analyzing correlated exposure biomarkers*

The advancement of exposure science in the past decade, particularly the expansion of the publically available biomonitoring datasets of the National Health and Nutrition Examination Survey (NHANES) generated by the US Centers for Disease Control and Prevention (CDC), have clearly demonstrated that the general population is exposed to many potential toxins simultaneously (Centers for Disease Control and Prevention and National Center for Health

Statistics 2003-2004; Rudel et al. 2003). Exposures to related compounds that share sources and routes of exposure are often highly correlated. However, conventional approaches to risk assessment and epidemiologic analyses of health effects have generally examined one chemical at a time. This can lead to many statistically significant predictors without informing the investigator of which are driving the association. A typical regression approach favoring parsimony might use selection techniques (e.g. stepwise) to subset predictors as either "in" or "out" with the latter being forced into a coefficient estimate of zero (Greenland 1994). In addition to possibly missing the combined impact of individually modest effects, selection algorithms have distorted uncertainty on parameter estimates, particularly those that are left out (Greenland 2000). The limitations of testing exposures one at a time without considering the mixture have been further demonstrated in experimental results that show that simultaneous exposures to estrogenic compounds that are individually below their no observed effect concentrations can have significant additive effects without interaction (Silva et al. 2002). In contrast with one at a time models, analytic techniques that model multiple correlated dosimeters simultaneously using maximum likelihood regression can lead to unstable effect size estimates. This problem of correlation of predictor variables in multiple regression is termed multicollinearity. An alternative approach for correlated predictors is to combine predictors to create summary score measures by manually fixing the additive weights applied to each component based on prior knowledge. Unfortunately, the resulting score doesn't allow estimation of the effects of individual components and prediction with such scores is sensitive to the weights that are selected. In addition to a loss of information, this often requires an assumption of additivity and a weighting scheme derived from laborious toxicologic work which must explicitly test the relative potency of each compound against a standard. Additional uncertainty in such weights arises from the use of experimental systems with subjective selection

of relevant endpoints as well as high-to-low dose and animal to human extrapolation (Kortenkamp 2007)

Because of all of these analytic challenges, an expert consensus report from the National Research Council called upon regulators to take new approaches to cumulative risk assessment and examine mixtures rather than considering environmental contaminants with common targets or modes of action one at a time (Committee on the Health Risks of Phthalates 2008). Further, the report recommended that new methods be developed using phthalates as a model system.

Bayesian approaches can estimate the relative weighting of each of the predictors within epidemiologic datasets. Details on the structure and theory of these models are discussed in detail in Chapter 5. Bayesian models have seldom been used for assessing effects of multiple exposures in the environmental health sciences, despite earlier calls for doing so (Greenland 1994). A noteworthy exception is a recent application of Bayesian hierarchical methods to the association between exposure to 18 herbicides and retinal degeneration under different modeling assumptions. However, the exposure information in this study was derived from a questionnaire and exhibited only moderate correlations that ranged from 0.06 to 0.58 (MacLehose et al. 2007). Although epidemiologists can use data augmentation for Bayesian modeling within popular software packages like SPSS and SAS (Greenland 2007), the widely available Bayesian inference Using Gibbs Sampling (WinBUGS) freeware makes more sophisticated models available for new applications without sophisticated programming (Lunn et al. 2000). Adapting these statistical models for the environmental health sciences and demonstrating improved performance over conventional methods could lead to their wider use and consequently address the gap in epidemiologic modeling techniques for multiple correlated exposures.

*Health outcomes: allergy and asthma burden in children*

The natural progression of childhood allergy and asthma is often referred to as the "atopic march" in which early eczema and atopy lead to allergic rhinitis and atopic asthma as a common pathway to childhood asthma (Leung et al. 2004; Zheng et al. 2011). Eczema, a chronic inflammatory skin disease characterized by pruritic rashes on the skin, can develop early in life with both allergic and other environmental triggers (Eigenmann et al. 1998; Leung et al. 2004; Novak and Bieber 2003; Novembre et al. 2001; Schmid-Grendelmeier et al. 2001). While eczema is often referred to as atopic dermatitis, as many as two-thirds of children with eczema are non-atopic in population based studies (Flohr et al. 2004). There is some evidence that eczema may predispose to allergic sensitization through disruption of epithelial barriers (Flohr 2011), which differs from a more classical characterization of eczema as an allergic disease but still would explain the stronger relation seen between severe eczema and multiple allergic sensitization (Novak and Bieber 2003; Schafer et al. 1999).

Incidence and prevalence of childhood allergic diseases and asthma increased substantially over the second half of the 20$^{th}$ century, particularly in westernized nations and their urban centers (Asher et al. 2006; Eder et al. 2006). This is partially attributed to increasing environmental exposures including to environmental tobacco smoke (ETS), aeroallergens, combustion byproducts, as well as decreased microbial exposure (Eder et al. 2006; Pearce et al. 2007). The varying prevalence of allergic diseases and asthma within ethnic groups and geographic regions and the increase in these diseases over a time span of decades both suggest the contribution of environmental factors. The asthma increase also corresponds temporally and geographically with the widespread production and use of phthalates in consumer products (Bornehag et al. 2004). The burden of disease is not evenly distributed; higher prevalence is noted in urban areas particularly in

poorer neighborhoods and among African American and Hispanic populations. A similar pattern is also reported for eczema in children with a higher prevalence in urban and African American households (Shaw et al. 2011). In 2000, asthma hospitalizations (which reflect disease severity and access to healthcare) among children in New York City were almost twice as high as those reported for the United States as a whole (Garg et al. 2003). In the CCCEH cohort, 26%, 25%, and 25% of children ages 5, 7, and 9 respectively have reported wheeze in the past 12 months on the ISAAC questionnaires.

*Airway inflammation*

FeNO is an easily collected marker of airway inflammation, a critical process in the pathogenesis and subsequent exacerbation of asthma. Higher FeNO precedes new-onset wheeze (Olin et al. 2010), and is higher among children with more frequent wheeze than less frequent wheeze (Cornell et al. accepted 2011). FeNO is also higher among atopic children and varies with exposure to allergen (Sordillo et al. 2011). Recent work has shown that FeNO measures collected at a single exhalation rate combine components of FeNO that are correlated with both atopy and wheeze that these are separable after collection at multiple flow rates (Rosa et al. 2011). FeNO has been shown to increase in both asthmatics and non-asthmatics after exposure to components of traffic related pollution as well as indoor pollutants such as formaldehyde (Cornell et al. accepted 2011; Eckel et al. 2011; Franklin et al. 2000; McCreanor et al. 2007). Because FeNO can be collected reliably in young children it is increasingly being utilized as a biomarker to study sub-clinical changes of airway inflammation in observational settings (Malmberg 2004).

*Epidemiologic evidence on phthalates, allergic disease, and respiratory health*

Recent epidemiologic studies suggest an association between home environments containing phthalates (measuring exposure indirectly) and increased symptoms of allergy and asthma in children. In a Finnish cross-sectional study based on parental report, children living in homes with plastic wall materials experienced a 3.42 (95% CI 1.13 to 10.36) increased odds of reported persistent wheeze (Jaakkola et al. 2000). Elevated odds ratios were also seen for report of cough and phlegm. In a Finnish case-control study of 251 bronchial-obstructed children with age-matched controls, children living in residences with PVC flooring had an OR of 1.89 (95% CI 1.14 to 3.14) relative to the reference category of wood or parquet flooring (Jaakkola et al. 1999). Both studies lack any direct phthalate exposure measurements.

Environmental levels of phthalates were measured in a Swedish case-control study comparing children with reported persistent eczema, rhinitis, or wheezing without a cold, to children without symptoms. Median concentrations of BBzP in bedroom dust were higher among cases than controls and in children with versus without physician-diagnosed rhinitis and eczema (Bornehag et al. 2004). Although rhinitis and eczema have both been classically considered allergic diseases (Flohr et al. 2004), there was no difference in the mean bedroom dust concentration of BBzP between atopic and non-atopic cases (Bornehag et al. 2004). PVC flooring was also significantly associated with case status. DEHP in dust was associated with physician-diagnosed asthma and showed a dose-response relationship across quartiles of exposure. In the adjusted analysis, the odds of physician diagnosed asthma were 2.93 (95% CI 1.36 to 6.34) times greater among those in the highest quartile of exposure compared to those in the lowest quartile (Bornehag et al. 2004). This study design was replicated in Bulgaria. Higher DEHP concentrations were found in bedroom dust of cases compared with controls with a dose-response in the odds of

being a case across quartiles of exposure. In the study in Bulgaria, BBzP was non-significantly higher in the dust of those reporting wheeze or eczema (Kolarik et al. 2008b).

There remains a lack of studies examining the association between biomarkers of phthalates and objective measures of children's pulmonary outcomes. In an NHANES III cross-sectional analysis examining adults, urinary metabolites of DBP and DEP were associated with decrements in pulmonary function tests only among males (Hoppin et al. 2004). A cross-sectional analysis in girls aged 6-8 in New York City found associations between metabolites of high molecular weight phthalates in urine and report of doctor diagnosed asthma and asthma related symptoms (Teitelbaum et al. 2008).

Two major limitations plague studies of phthalate exposure and respiratory outcomes (see our review (Kwak et al. 2009) and others (Jaakkola and Knight 2008; Mendell 2007; Nielsen et al. 2007)). First, indirect exposure measures such as recording the presence of materials in the home or measuring phthalates in house dust (Bornehag et al. 2004; Jaakkola et al. 1999; Jaakkola et al. 2000; Kolarik et al. 2008b), can lead to misclassification of exposure and likely would bias studies toward the null. Second, the lack of a prospective design prevents determination of causality. Retrospective or cross-sectional studies lack temporal ordering of exposures prior to disease. Therefore they cannot preclude, for example, that observed associations may be due to reverse causality (Bornehag et al. 2005b). This order of events might plausibly occur if parents of asthmatic children attempted to reduce the presence of airborne triggers for asthma by replacing carpets with vinyl flooring, a source of phthalates.

*Toxicologic evidence on phthalates, allergy, and respiratory health*

Although the proposed mechanism for the role of phthalates in asthma and respiratory health is still unclear, the strongest toxicologic evidence suggests some phthalates, particularly DEHP, may act as adjuvants (but not intrinsic allergens (Butala et al. 2004)) enhancing the immune and allergic response (Larsen et al. 2002). Phthalates may contribute to airway inflammation by enhancing IgE-mediated type I hypersensitivity to common aero-allergens. Two inhalation adjuvant studies on BALB/cJ mice found increases in IgG1 but not IgE after coexposure to ovalbumin and either inhaled DEHP or its metabolite MEHP (Hansen et al. 2007; Larsen et al. 2007). IgG1 is often used as a surrogate marker for Th2 responses in mice which regulate it similarly to IgE (Dearman et al. 2009). In a different strain of atopic prone mice, splenocytes stimulated ex-vivo with DEHP have enhanced differentiation and Th2 type responses (secretion of IL-4) (Koike et al. 2009). In BALB/c mice first primed with a novel antigen, keyhole limpet hemocyanin (KLH), and alum, the ex-vivo addition of DEHP or another phthalate DiNP with KLH enhanced Th2 type responses (IL-4 and not IFN-γ) in a dose-dependent fashion in lymph node cells. The experiment showed that this was due to CD4+ T cells which had upregulated IL-4 gene expression. This resulted in dose-dependent enhanced production of total and KLH specific IgE for both phthalates compared to KLH stimulation alone (Lee et al. 2004). Incubating human peripheral blood mononuclear cells with DEHP or its metabolite MEHP ex-vivo potentiated an increased histamine release (2-3x) after stimulation with another allergy promoting factor and the addition of cross-linking anti-IgE compared with controls (Glue et al. 2005). DEHP and DBP also act as adjuvants enhancing the development of atopic dermatitis with exposure to dust mites (Yanagisawa et al. 2008) or a chemical hapten (Shigeno et al. 2009) in two mouse models of

allergy formation. In contrast with these other phthalates, BBzP does not appear to have adjuvant effects in ovalbumin sensitization animal models (Dearman et al. 2009; Larsen et al. 2003).

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**Chapter 3: Urinary and air phthalate concentrations and self-reported use of personal care products among minority pregnant women in New York City**

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**Abstract**

Diethyl phthalate (DEP) and di-*n*-butyl phthalate (DnBP) are used extensively in personal care products, including fragrances (DEP) and nail polish (DnBP). Between May 2003 and July 2006, we gathered questionnaire data on use of 7 product categories (deodorant, perfume, hair spray, hair gel, nail polish/polish remover, liquid soap/body wash, lotion/mist) over 48 hours during the 3$^{rd}$ trimester of pregnancy from 186 inner-city women. A 48-hour personal air sample was collected and analyzed for DEP and DnBP; a maternal spot urine sample was collected and analyzed for their monoester metabolites, monoethyl phthalate (MEP) and mono-*n*-butyl phthalate (MnBP), respectively. Ninety-seven percent of air samples and 84% of urine samples were collected within ±2 days of the questionnaire. During the 48 hours, 41% of women reported perfume use and 10% reported nail polish/polish remover use. In adjusted analyses, no association was seen between nail product use and air DnBP or urine MnBP concentrations. Women reporting perfume use had 2.3 times higher (95% CI 1.6, 3.3) urinary MEP concentrations. Personal air DEP increased 7% for each 25% increase in a composite indicator of the 6 other product categories ($p<0.05$) but was not associated with perfume use. Air DEP was correlated with urine MEP concentrations only among non-perfume users ($r=0.51$, $p<0.001$). Results suggest that perfume use is a significant source of DEP exposure.

**Keywords:** diethyl phthalate (DEP), di-*n*-butyl phthalate (DnBP), human biomonitoring, personal care products

## Introduction

Phthalates are diesters of phthalic acid that are commonly used in a wide variety of consumer products. Human exposures come from their use in toys, household materials, medical devices, in the processing and packaging of foods, and personal care products (Schettler 2006). Some phthalates are under increasing scrutiny in epidemiologic studies examining potential associations with adverse reproductive and developmental outcomes including changes in gestational age, urogenital tract development, sperm quality, and asthma among other endpoints (Swan 2008). However, relatively few studies have examined the relation between sources, exposure pathways, and internal dosimeters.

Two phthalates, diethyl phthalate (DEP) and di-*n*-butyl phthalate (DnBP), are added as a solvent for fragrances or to prevent products from becoming brittle, and have been found at higher concentrations than other phthalates in testing of personal care products in the United States, South Korea, and China (Houlihan et al. 2002; Hubinger and Havery 2006; Koo and Lee 2004; Shen et al. 2007). Among personal care products, DEP and DnBP have been found at the highest concentrations in fragrance products, including perfume (DEP), and in nail polishes (DnBP). Figure 1 shows an adaptation of results from the analysis of DEP in 48 personal care products in the United States (Hubinger and Havery 2006). The five fragrance products tested had concentrations of DEP ranging from 5486 to 38663 ppm, and the next highest DEP concentration of any other product tested was in a deodorant with 2933 ppm (Hubinger and Havery 2006). In these data, fragrances have consistently higher concentrations of DEP compared with all other products tested, supporting the separate analysis of perfume from other personal care product categories as potential sources of DEP. According to a review of patent records, nail polishes might contain 50000 ppm (5%) DnBP (Houlihan et al. 2002), a finding that was supported by a

study that tested six nail enamel products and found concentrations that ranged from below the limit of detection to 59815 ppm, or roughly 6% (Hubinger and Havery 2006). Thus, nail polishes should be analyzed separately from other categories of personal care products as potential sources of exposure to DnBP because nail polishes appear to be more likely to contain DnBP and at higher concentrations than other product categories. Under current regulations in which ingredients used in fragrances are exempt from disclosure, phthalates are not generally listed as ingredients on consumer products in the United States (Steinemann 2009).

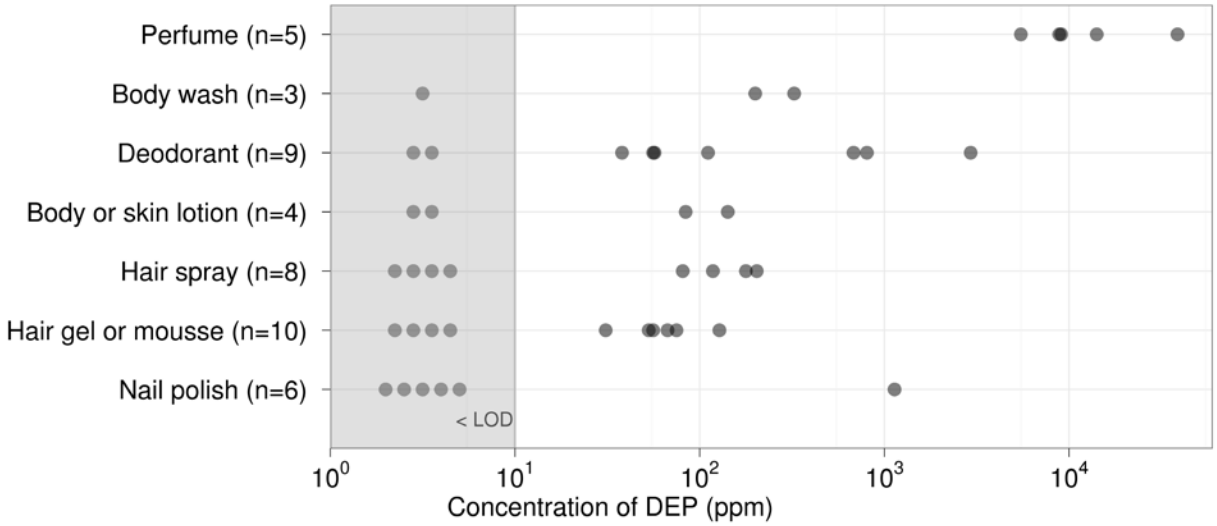


**Figure 1. Diethyl phthalate in personal care products**
Adapted from Table 2 of Hubinger and Havery, 2006. A total of 48 products purchased in the Washington, DC area were tested. Non-detectable values are displayed as less than the limit of detection of 10ppm. Hand cream (n=2) and shampoo (n=1) are not shown. No direct product testing took place in the current study.

Phthalates can enter the body via ingestion, dermal absorption, parenteral intake from medical devices, and inhalation. They undergo rapid hydrolysis to monoesters; short-alkyl chain phthalates like DEP and DnBP are principally excreted in the urine as hydrolytic monoesters or as their corresponding glucuronidated conjugates (Silva et al. 2003). In 16 human volunteers who

ingested a labeled dose of DnBP, 69% was excreted as mono-*n*-butyl phthalate (MnBP) in urine with undetectable levels of urinary MnBP after the first 24 hours (Anderson et al. 2001). Half-lives of DEP were similarly short in animal studies (Api 2001). Both DEP and DnBP are also taken up dermally with an estimated 6% and 2% respectively excreted as their urinary metabolites monoethyl phthalate (MEP) and MnBP by human volunteers after dermal application (Janjua et al. 2008). The majority of dermally absorbed DEP was excreted within 8 hours and MnBP excretion lagged slightly compared to MEP but was largely excreted within 24 hours. Urinary MEP concentrations from a representative sample of the US population (National Health and Nutrition Examination Survey [NHANES] 1999-2000) were highest at a midday collection which was hypothesized to be related to the application of personal care products in the morning (Silva et al. 2004).

Some evidence already exists for an association between frequency of personal care product use and urinary concentrations of phthalates. Men reporting the use of cologne or aftershave over 48 hours had higher urinary MEP concentrations than other men (Duty et al. 2005). Another study reported an association between the use of baby care products and concentrations of MEP and two other phthalate metabolites but not MnBP in infant urine samples (Sathyanarayana et al. 2008). An increase in the number of personal care products used in the previous 48 hours was associated with higher urinary MEP in 19 pregnant women in Israel (Berman et al. 2009). Work as a manicurist in a nail-only salon was associated with urinary concentrations of MnBP in two occupational studies among nail-only salon workers. Urinary MnBP concentrations post-shift were 49% higher than pre-shift in 37 manicurists from Massachusetts in 2004-2005 (Kwapniewski et al. 2008). In 26 manicurists in Maryland studied between 2003 and 2005, those using gloves had 50% lower post-shift MnBP concentration compared to non-users (Hines et al. 2009). Collectively,

these five studies indicate the potential importance of dermal absorption for exposures to DEP and DnBP and the suitability of urinary metabolites to assess these exposures.

We have reported previously that DEP and DnBP and their metabolites were found in 100% of personal air and urine samples collected from inner-city women during pregnancy (Adibi et al. 2008). The purpose of the current study was to determine if personal care product use was associated with measures of phthalate exposure in air and urine samples among the same urban cohort of pregnant women. To do this, we evaluated the relationship between self-reported prenatal personal care product use and concentrations of DEP and DnBP in personal air samples and MEP and MnBP in urine samples. The particular focus was on perfume and nail product use and exposures to DEP and DnBP, respectively.

## Methods

### *Study subjects*

Participants (n = 186) were selected from the Mothers and Newborns cohort study of the Columbia Center for Children's Environmental Health (CCCEH) based in Northern Manhattan and the South Bronx, New York (Perera et al. 2003; Whyatt et al. 2003). Selection was based on the availability of a product use questionnaire and phthalates measured within a week in either a personal air and/or urine sample collected during the third trimester of pregnancy. Overall 97% of air samples and 84% of urine samples were collected within two days of the product-use questionnaire. In most cases there was no difference when analysis was limited to the subsets within two days and results are given for the whole cohort unless otherwise specified. Enrollment criteria for the CCCEH cohort have been described elsewhere (Perera et al. 2003; Whyatt et al. 2003). The study was restricted to women 18-35 years old who self-identified as either African

American or Dominican and had resided in Northern Manhattan or the South Bronx for at least one year prior to pregnancy. Women were excluded at enrollment if they reported that they smoked cigarettes or used other tobacco products during pregnancy, used illicit drugs, had diabetes, hypertension or known HIV, or had their first prenatal visit after the 20th week gestation. Study procedures, including questionnaires, personal air monitoring and collection of biologic samples, were explained to each subject at enrollment and a signed consent, approved by the IRB of Columbia University and the Centers for Disease Control and Prevention (CDC), was obtained.

*Product use questionnaire*

A brief questionnaire that was administered in the third trimester (mean gestational age 35 weeks) asked participants to recall their use of various types of personal care products over the previous 48 hours and throughout the individual trimesters of pregnancy. They were asked about use (yes/no), the number of total uses over 48 hours, and the frequency of use during each trimester (>1/day, 1/day, 2-3/week, 1/week, <1/week-1/month, <1/month). From the questionnaire, we selected seven product categories for this analysis: deodorant, lotion or mist (spray application), perfume, liquid soap or body wash, hair gel, hair spray, and nail polish or polish remover. Because the questionnaire asked about nail polish or polish remover together, we refer to this category as nail products. The product categories selected were those likely to contain DEP or DnBP and which were used by ≥ 10% of participants. Six and 20 participants were missing information on the frequency of use of product categories in the 48 hour period and third trimester, respectively.

*Sample collection and analysis*

Participants carried backpacks for 48 hours containing pumps drawing personal air samples at 4 L/min from near the breathing zone onto a quartz filter with a polyurethane foam cartridge backup. Air samples were stored in a freezer and shipped on dry ice to Southwest Research Institute (San Antonio, Texas) for extraction and analysis of phthalates via gas chromatography / mass spectrometry (Adibi et al. 2008). Laboratory matrix blanks were extracted and analyzed with each batch of samples, to assess laboratory-introduced phthalate contamination. Although DEP and DnBP were often detected, concentrations in the laboratory blanks (n=53) were substantially lower than personal air extracts (average of 993 ± 2617 versus 24838 ± 23047 ng/extract for DEP and 288 ± 263 versus 6325 ± 5963 ng/extract for DnBP). Personal air samples were collected for 168 of 186 participants (90%).

A spot urine sample was collected generally at the start or conclusion of the 48 hour personal air monitoring. The date, but not exact time of collection, was available. The urinary concentrations of nine phthalate metabolites, including MEP and MnBP, were measured at the National Center for Environmental Health, CDC. Urine samples underwent an enzymatic deconjugation reaction followed by solid-phase extraction; the phthalate metabolites were separated with high-performance liquid chromatography, and detected with isotope-dilution tandem mass spectrometry as described previously (Adibi et al. 2008; Kato et al. 2005). The limits of detection (LODs), which varied slightly depending on the method used, were in the low ng/ml range. Specific gravity was measured at room temperature at the CDC with a handheld refractometer. Urinary concentrations were adjusted for dilution in statistical analysis using a formula from Hauser et al. adapted with a constant that is more appropriate for urinary dilution during pregnancy (Hauser et al. 2004; Teass et al. 1998). The constant value is derived from the

median specific gravity of pregnant women in the CCCEH cohort study. The formula is $P_c = P[(1.016-1)/(SG-1)]$, where $P_c$ is the specific-gravity-corrected phthalate concentration, P is the observed phthalate concentration and SG is the specific gravity of the urine sample. Urine samples were collected for 164 of 186 participants (88%).

Air and urine samples were collected between May 2003 and July 2006. Extracts from air samples were analyzed between January 2006 and November 2007. Urine samples were analyzed between 2004 and 2007.

*Statistical methods*

For personal air concentrations of DEP and urinary concentrations of MEP, perfume use was examined separately from the other products. We aggregated data on other products used by summing the reported number of product uses over the 48 hour period for the other six product categories. For analytical purposes we then assigned study subjects to quartile categories across the distribution of summed product uses. For example, a participant reporting, of the six categories, one use of hair gel, one use of liquid gel, two uses of lotion, and two uses of deodorant would have six total uses which would put them in the second quartile for use of other products. For analyses of DnBP and MnBP, nail products were examined separately and the use of other products was similarly summed and classified into quartiles. All statistical tests on urinary and air measures were conducted with natural logarithm transformed concentrations after adjustment of urine data for specific gravity. Assumptions of normality were assessed visually with quantile-quantile plots. Correlation tests used Pearson's correlation coefficient. Simulation using informal Bayesian inference with uniform priors was used to graph uncertainty in regression parameters. For visual interpretation, this displays ~95% of simulation draws of regression slopes within two standard

errors for each parameter estimate (Gelman and Hill 2007). Differences in group means were assessed with Student's t-test or with a multiple partial F-test for indicator variables in multivariable linear regression when controlling for covariates. Model building began with unadjusted models including only exposure variables derived from the product use questionnaire. The full model included demographic covariates, selected to be consistent with prior research, for race/ethnicity, age, education, and BMI that might contribute to confounding or increase explanatory power (Duty et al. 2005). Unadjusted models had similar results to the multivariable results presented here. The quartiles of product use variables were first assessed with a series of three indicator variables relative to the lowest quartile to evaluate the assumption of monotonicity and then if appropriate used in multivariable regression as a continuous measure. Parameter estimates from regression were exponentiated and presented as fold-changes. The coefficient of determination, $R^2$, was examined as the proportion of the variance in the outcome explained by the linear model. All statistical analyses were conducted in R version 2.9.1 (R Development Core Team 2011).

## Results

### *Sample size, demographics, and reported product use*

Participant characteristics for the 186 women are detailed in Table 1. Those who were missing an air or urine sample did not differ on demographic characteristics from the remainder.

**Table 1.** Characteristics of 186 pregnant study participants

| | |
|---|---|
| **Age (years)**[1] | 26 ± 5 |
| **Ethnicity (%)** | |
| African American | 28 |
| Dominican or other Hispanic | 72 |
| **Education (%)** | |
| High school diploma, GED, or greater | 61 |
| **Body Mass Index**[1] | 27 ± 7 |
| **Maternal ETS** | |
| % reporting smoker in the home | 25 |
| **Reported use (yes versus no) of categories of personal care products over a 48 hour period (%)** | |
| Deodorant | 98 |
| Lotion | 82 |
| Perfume | 41 |
| Liquid soap | 29 |
| Hair gel | 25 |
| Hair spray | 10 |
| Nail polish or polish remover | 10 |

[1]Mean ± standard deviation

*Product use*

Participants reported using an average of 3 product types of the seven categories in this analysis. The median for the total number of times the women used any product was 7 with a range from 1 to 26 (n = 180). Table 1 lists the frequency of reported use of the product categories in the 48 hour period. Deodorant was the product category with the most prevalent use (98%). The most frequently used product category was liquid soap (mean of 3.4 uses in 48 hours among participants reporting use of that product), followed by lotion and deodorant. Perfume use in the 48 hour period of the questionnaire was reported by 41% of participants and was higher among African Americans (45%) than among Dominicans (40%) although the difference in proportions was not significant. Perfume users had a median of 2 reported uses over the 48 hour period. Overall, 84% reported using perfume at some point throughout their pregnancy and those reporting usage in the

48 hour period during the third trimester, were more likely to report having used perfume in the first two trimesters (chi-squared 17.2 $_{1 \text{ degree of freedom (DF)}}$, $p < 0.01$). In the 166 participants with information about frequency of use in the third trimester, 61 reported using perfume at least daily (37%). The proportion reporting at least daily use in the third trimester was higher among those reporting perfume use in the 48 hour period (63%) compared with those not reporting perfume use in the 48 hour period (17%) (chi-squared 34.4 $_{1 \text{ DF}}$, $p < 0.001$). There was no association between perfume use (yes/no) and quartiles of the total uses of the other six product categories (n = 180, chi-squared 1.4 $_{3 \text{ DF}}$, $p = 0.7$). Use of nail products over the 48 hours was reported by 10% of participants (18 of n = 186). Nail product users all reported a single use over the 48 hour period. Overall, 69% of participants reported using nail products at some point throughout their pregnancy and those reporting usage in the 48 hour period in their third trimester were more likely to report having used nail products in the first two trimesters (chi-squared 7.2 $_{1 \text{ DF}}$, $p < 0.01$). There was no difference in the quartile of the sum of product uses between African American and Dominican participants (n = 180, chi-squared 0.5 $_{3 \text{ DF}}$, $p = 0.92$).

*Personal air and urinary metabolite concentrations*

DEP and DnBP were detected in 100% of air samples (n = 168) and MEP and MnBP were detected in 100% of urine samples (n = 164). The distribution of phthalates in personal air and metabolites in urine are summarized in Table 2. We did not see a temporal trend from 2003 to 2006 in concentrations of DEP and DnBP in personal air samples or MEP and MnBP in urine in a visual display using a lowess plot (data not shown).

**Table 2.** Distribution of phthalate diester concentrations in personal air (ng/m$^3$) and metabolite concentrations in urine (ng/mL)

| | | | Percentile | | | | | |
|---|---|---|---|---|---|---|---|---|
| Phthalate diester$_1$ | Phthalate metabolite$_2$ | Percent > LOD | 5th | 25th | 50th | 75th | 95th | GM (95% CI) |
| (ng/m$^3$) | | | | | | | | |
| DEP | | 100 | 747 | 1276 | 1730 | 2532 | 4346 | 1816 (1668 to 1977) |
| DnBP | | 100 | 206 | 310 | 449 | 626 | 1077 | 459 (421 to 499) |
| | (ng/mL) | | | | | | | |
| | MEP | 100 | 37 | 103 | 199 | 489 | 3184 | 243 (198 to 298) |
| | MnBP | 100 | 6 | 20 | 36 | 84 | 203 | 38 (32 to 45) |

GM, geometric mean; LOD, limit of detection

$_1$Personal air concentrations of phthalates were available for n = 168.

$_2$Urinary metabolite concentrations of phthalates were available for n = 164.

Air and urine concentrations of phthalates and their metabolites were correlated. The correlation of DEP and MEP (n = 146, r = 0.36, $p < 0.001$) was similar to the correlation for DnBP and MnBP (r = 0.32, $p < 0.001$). Correlations were similar when restricted to urine samples collected within ±2 two days of the conclusion of the 48 hour personal air sample (n = 126, DEP and MEP, r = 0.36, $p < 0.001$; DnBP and MnBP, r = 0.31, $p < 0.001$). The concentrations of the two metabolites MEP and MnBP were also correlated (r = 0.40, $p < 0.001$) as were the concentrations of the two parent compounds, DEP and DnBP, in the personal air samples (r = 0.33, $p < 0.001$). There appeared to be no correlation between DEP and MnBP (r = 0.04, p = 0.62) or between DnBP and MEP (r = 0.11, p = 0.20).

African Americans had higher concentrations of DEP in their personal air with a geometric mean that was 56% higher than among Dominicans (t-test, $p < 0.001$). The adjustment for individual product categories, including perfume, or for counts of the number of categories of potentially DEP containing product types or categories of other hair products did not explain this difference (data not shown). Although urinary concentrations of MEP were higher among African

Americans than Dominicans (geometric mean 55% higher, t-test, p=0.07), the difference was of borderline significance.

Results from the adjusted analyses of the relationship between product use and both DEP in personal air and MEP in maternal urine are presented in Table 3. There was no association between perfume use and air DEP concentrations. However, DEP increased by 7% for each quartile increase in the sum of uses of the other six products ($p < 0.05$). Perfume use was significantly associated with MEP concentration in urine samples. Specifically, women reporting perfume use in the 48 hour questionnaire period had 2.3 times higher concentrations of urinary MEP than those not reporting use in the same period (95% CI 1.6 to 3.3, $p < 0.001$). Further, controlling for perfume use, there was a significant association between quartiles of use of the other six products and urinary MEP concentration (a 26% increase in MEP concentrations for each quartile increase in the sum of product uses, $p < 0.01$). The full model explains 19% of the variance in urinary MEP. To further evaluate the dose-response relationship between perfume use and urinary MEP, analyses were restricted to subjects with urine collected within two days of the questionnaire ($n = 128$). Results are presented in Figure 2 and show a dose-response relation between urinary MEP and reported number of times perfume was used over the 48-hour period after adjustment for race/ethnicity (t-test on regression coefficient for continuous measure, $p < 0.001$). We also examined the correlation between DEP in air and MEP in urine in the same subset of study participants. Interestingly, among perfume users there was no correlation ($n = 57$, $r = 0.12$, $p = 0.36$). However, among non-perfume users, the correlation was highly significant and stronger than seen for the full cohort ($n = 69$, $r = 0.51$, $p < 0.001$). Results are displayed in Figure 3.

**Table 3.** Multivariable regression results for association of product use and covariates with personal air DEP and urine MEP concentrations

| | **DEP** (n = 163; $R^2$ = 0.19) | | **MEP** (n = 160; $R^2$ = 0.19) | |
|---|---|---|---|---|
| | **Fold change (95% CI)** | | **Fold change (95% CI)** | |
| Perfume use[1] | 1.09 | (0.9 to 1.3) | 2.29 | (1.6 to 3.3)*** |
| Quartile of use of other products[2] | 1.07 | (1.0 to 1.2)* | 1.26 | (1.1 to 1.5)** |
| Race/ethnicity[3] | 1.53 | (1.3 to 1.8)*** | 1.22 | (0.8 to 1.8) |
| Age (years) | 0.99 | (1.0 to 1.0) | 0.98 | (0.9 to 1.0) |
| Education[4] | 0.96 | (0.8 to 1.1) | 1.31 | (0.9 to 1.9) |
| BMI[5] | 1.00 | (1.0 to 1.0) | 1.02 | (1.0 to 1.0) |

*p-value < 0.05; ** < 0.01; *** < 0.001

[1] 0 = no use in previous 48 hour period; 1 = yes. [2] quartiled sum of uses in 48 hour period of deodorant, lotion, liquid soap, hair gel, hair spray, and nail polish/remover. [3] 0 = Dominican; 1 = African American. [4] 0 = no high school degree or equivalent; 1 = high school degree or greater. [5] Pre-pregnancy body mass index (kg/$m^2$).

Parameter estimates are exponentiated to aid interpretability as a multiplicative fold change.

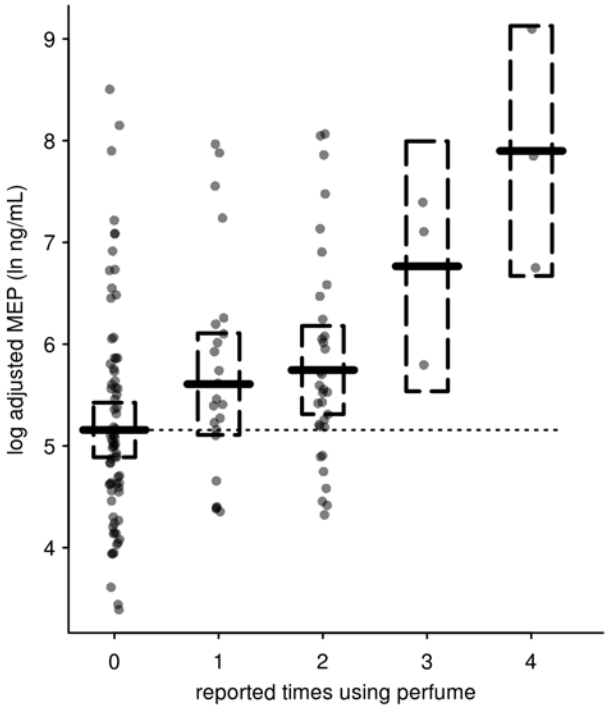


**Figure 2. Log urinary MEP concentrations (adjusted for specific gravity) for participants with 0 to 4 reported uses of perfume over a 48 hour period and samples collected within two days of the questionnaire controlling for race/ethnicity (n = 137).**
Group means shown with dashed rectangles indicating 95% CI. The dotted line extends the group mean for non-users. The group means for those using perfume 2, 3, or 4 times are all higher than non-users with single users of borderline significance (p = 0.08).

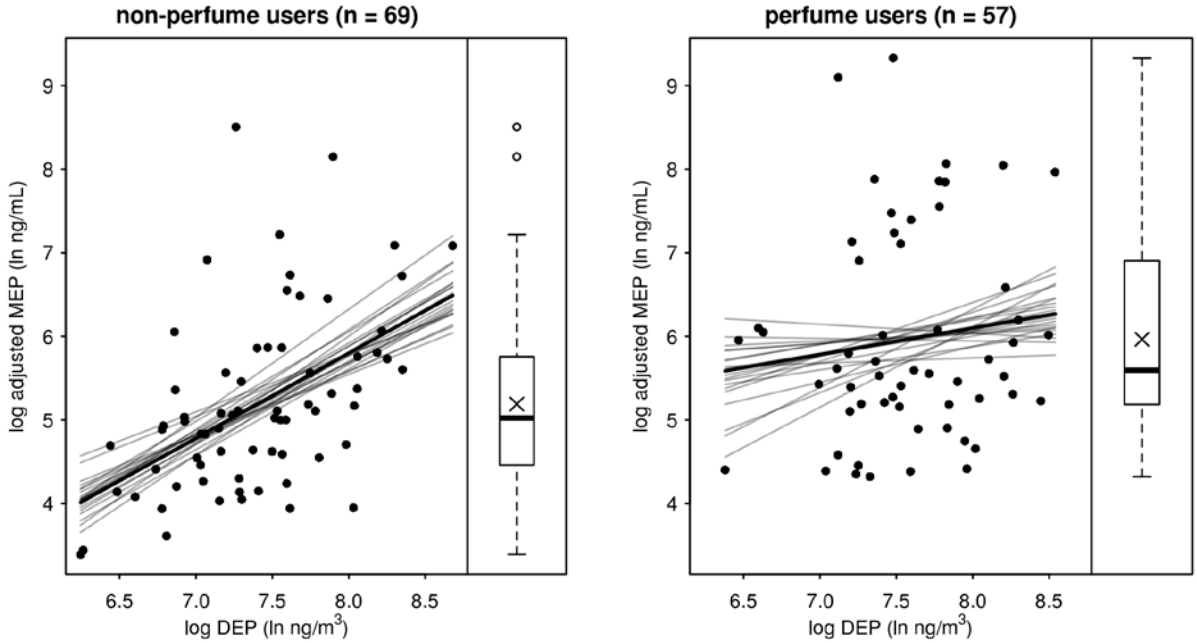


**Figure 3. Association between DEP concentrations in personal air and the urinary metabolite MEP concentrations (adjusted for specific gravity) stratified by perfume use using linear regression of log transformed values.**
Lighter lines represent predictive uncertainty in regression parameters from informal Bayesian simulations (20 simulation draws with uniform priors). Boxplots show the distribution of MEP with means ("X").

Participants reporting use of nail products had no differences in DnBP concentrations in their personal air (n=16) compared with non-users (n=152), (geometric mean and 95%CI, 392 (292 to 521) versus 466 (427 to 510) ng/m$^3$ DnBP). Similarly, those reporting use had no differences in MnBP concentrations in their urine (n=16) compared with non-users (n=148) (geometric mean and 95% CI, 42 (27 to 64) versus 39 (34 to 45) ng/ml). As shown in Table 4, there was no association between reported use of nail products or the composite indicator of the use of other products and either DnBP in personal air or MnBP in urine. Additionally, none of the other covariates were significant predictors of either DnBP or MnBP concentration and the regression models explained $\leq$ 5% of the variance in DnBP and MnBP (see Table 4).

**Table 4.** Multivariable regression results for association of product use and covariates with personal air DnBP and urine MnBP concentrations

| | **DnBP** (n = 163; $R^2$ = 0.05) | | **MnBP** (n = 160; $R^2$ = 0.04) | |
|---|---|---|---|---|
| | **Fold change** | **(95% CI)** | **Fold change** | **(95% CI)** |
| Nail polish/ remover use[1] | 0.84 | (0.6 to 1.1) | 1.08 | (0.7 to 1.7) |
| Quartile of use of other products[2] | 1.05 | (1.0 to 1.1) | 0.96 | (0.9 to 1.1) |
| Race/ethnicity[3] | 0.95 | (0.8 to 1.2) | 0.78 | (0.6 to 1.1) |
| Age | 0.99 | (1.0 to 1.0) | 0.98 | (1.0 to 1.0) |
| Education[4] | 0.87 | (0.7 to 1.1) | 1.05 | (0.8 to 1.4) |
| BMI[5] | 1.00 | (1.0 to 1.0) | 1.02 | (1.0 to 1.0) |

$^{*}$p-value < 0.05; $^{**}$ < 0.01; $^{***}$ < 0.001

[1] 0 = no use in previous 48 hour period; 1 = yes. [2] quartiled sum of uses in 48 hour period of deodorant, lotion, liquid soap, perfume, hair gel, and hair spray. [3] 0 = Dominican; 1 = African American. [4] 0 = no high school degree or equivalent; 1 = high school degree or greater. [5] Pre-pregnancy body mass index (kg/m$^2$).

Parameter estimates are exponentiated to aid interpretability as a multiplicative fold change.

## Discussion

The use of personal care products was common during pregnancy among women in this urban cohort study. Participants used multiple products and many used perfume on a daily basis in the third trimester of pregnancy. Nail product use, while common throughout pregnancy, was less frequent than other categories of personal care product use.

Perfume use over a 48 hour period was associated with increased concentrations of MEP, the urinary metabolite of DEP. Women who reported using perfume had on average 2.3 times higher concentrations of urinary MEP after adjustment for urinary dilution and covariates in a multiple regression analysis. Our results further show a significant dose-response relationship between the number of perfume uses in a 48 hour period and urinary concentration of MEP. Increased use of other product types (deodorant, lotion or mist, liquid soap or bodywash, hair gel,

hair spray, and nail products) was associated with higher concentrations of DEP in air and MEP in urine.

While perfume use was associated with urinary MEP, we found no association between use and DEP in personal air sample. This lack of association was unexpected as we had hypothesized that DEP would volatilize during perfume use and contribute to inhalation exposures. It is entirely possible that the lack of association is a result of limitations in the available dataset. However, another possible explanation is that the contribution of perfume use to total exposure comes more from dermal uptake than inhalation. Further, if perfumes, which have higher concentrations of DEP than other personal care products, contributed substantially to total exposure and did so primarily via dermal uptake, it might explain why there was no correlation between air DEP and urinary MEP among perfume users. In contrast, among non-perfume users there was a relatively strong correlation between air DEP and urinary MEP suggesting that the backpack monitors were useful in measuring exposures via inhalation. This association, although not a pharmacokinetic model to quantify the mass-balance contribution of inhalation, is consistent with inhalation as a pathway of exposure to some product categories. A shorter time frame of exposure monitoring, coordinated to capture episodic events, might have been a better sampling design for detecting associations with episodic high exposures. However, these data are not generally available in observational studies, including in the current cohort, in which 48 hour personal air samples were collected in order to characterize the exposure profile of the late pregnancy period.

The classification of participants into quartiles of the use of non-perfume product types serves as a crude indicator for some of these sources (particularly personal care products). This approach increased our statistical power to detect associations; however at the expense of

sensitivity to identify specific sources. The combined variable was positively associated with both DEP in personal air and MEP in urine. In comparing personal air and urinary exposure, it is important to note that participants were told to keep the backpack monitor out of the bathroom while they showered to avoid humidity that could damage the pump. We cannot discount that this may have had an impact on the association between product use and the collection of personal air samples. However, the association of the composite indicator for non-perfume product types such as hair spray and lotion, which are also products likely to be used in the bathroom, with DEP in personal air suggests that personal air monitoring was sampling exposure events resulting from the use of personal care products. Perfume use may represent a greater contributor to DEP exposure than other personal care products. This is consistent with the substantially higher detection frequencies and concentrations of DEP found in perfumes compared with other product types (Houlihan et al. 2002; Hubinger and Havery 2006; Koo and Lee 2004; Peters 2005; Shen et al. 2007). However, reports of perfume use, even among those with a daily use pattern, may be too imperfect a measure of exposure to detect differences in personal air DEP given the limitations of this dataset.

There was no association between reported use of nail products or the quartiles of usage of other products over the previous 48 hours with concentration of either DnBP in personal air or MnBP in urine samples. Further, our study did not identify any significant predictors of DnBP or MnBP concentration. However, the proportion of nail product users was small (10%) and our questionnaire was designed to characterize use patterns and grouped the use of nail polish and polish remover together. In addition, DnBP concentrations in nail polish may vary in concentration more than that of DEP in perfume. In one study that sampled six off-the-shelf nail enamel formulations in the United States, the concentrations of DnBP ranged from less than 10 to 59815

ppm (Hubinger and Havery 2006). In addition, some products are being reformulated to remove phthalates and the prevalence of phthalates in nail polish may be changing (Hubinger and Havery 2006). Thus, a questionnaire alone might be insufficient to assess potential exposure to DnBP among personal users of these products. In contrast, two occupational studies of nail-only salon workers found associations between shift work and exposure to DnBP as measured by urinary MnBP (Hines et al. 2009; Kwapniewski et al. 2008).

We have reported previously on the distribution of phthalate concentrations in personal air and metabolites in urine (Adibi et al. 2008). Although the personal air results presented here are on a larger sample (168 versus 96 women), the distributions of DEP, DnBP, MEP, and MnBP are entirely consistent with our previous results which showed that on average concentrations of MEP were similar in this population to those in NHANES (females 18-40 years of age in the 1999-2000 & 2001-2002 NHANES) but that participants in the CCCEH had higher urinary concentrations of MnBP (Adibi et al. 2008). Our understanding of the previously reported correlation between personal air DEP and urinary concentrations of MEP is enhanced by stratifying by the use of perfume. Among non-perfume users there is a higher correlation than we have previously reported and among perfume users there is no apparent association between DEP and MEP.

Our questionnaire covered only a subset of potential products used in the home which might contain phthalates. Use of products in these categories could be indicative of a preference for products containing fragrance and perhaps use of other phthalate-containing products as well, such as air fresheners or cleaning products. In addition, our questionnaire did not include the amount of products used which can vary substantially; one study, for example, found an 18-fold range in the average mass of spray perfume used per day among regular female users over a two week period (Loretz et al. 2006). Individuals also vary in their uptake of phthalates after exposure, in

metabolism of the parent compound into the urinary metabolites, and in the timing of their urine sample relative to product use. We would expect additional contributors to this type of variability among pregnant women due to differences in gestational age at the time of sampling, blood volume, and renal and placental function (Adibi et al. 2008). All of these unmeasured factors contribute to variability and the limited explanatory power of the models presented here. While the multivariable regression model presented in Table 3 explained 19% of the variability in urinary MEP, the $R^2$ from a univariate model of perfume use (yes/no) alone, while highly significant, only explained 11% of the variance in the specific gravity adjusted and log transformed urinary concentrations of MEP. Both our multivariable and univariate regression models explained $\leq 5\%$ of the variance in DnBP or MnBP.

In conclusion, we report that pregnant women in this urban cohort used multiple personal care products and that the use of perfume was positively associated with urinary concentrations of MEP, a surrogate measure of exposure to DEP. It is important to assess if there are adverse effects of human exposures during critical periods given the heightened exposure to DEP associated with the common use of personal care products.

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**Disclaimer**

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the CDC.

**Conflict of Interest**

The authors declare no conflict of interest.

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**Chapter 4: Vinyl flooring in the home is associated with airborne residential butylbenzyl phthalate and urinary metabolite concentrations**

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**Abstract**

Background and Aims: Phthalate plasticizers are found in consumer products and home furnishing materials but their contribution to personal exposure remains unclear. Previous studies have shown associations between vinyl flooring and plastic wall coverings and house dust concentrations of both butylbenzyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP), however total exposure to the latter is believed to be primarily dietary. We hypothesized that children living in homes with vinyl flooring would have higher concentrations of BBzP in their home indoor air and higher concentrations of monobenzyl phthalate (MBzP, a BBzP metabolite) in their urine than other children.

Methods: BBzP and DEHP exposures were examined in an ongoing prospective cohort of New York City children 5-10.6 years old (*n*=220) using: (1) a checklist for the presence of potential phthalate containing materials in the home (including "vinyl or linoleum flooring"); (2) a two-week home indoor air sample collecting particle and gas phase phthalates at 1.5 LPM at the child's breathing height; and (3) urinary metabolites analyzed in a spot sample (*n*=176 collected during air sampling).

Results: The category "vinyl or linoleum" flooring was observed in 125 (57%) of the homes. Homes with vinyl or linoleum flooring had a significantly higher concentration of BBzP (Geometric mean (GM) 24.3; interquartile range [IQR] [9.3, 66.8] ng/m$^3$), than those with wood or carpet flooring (GM 10.9; IQR [6.9, 14.1] ng/m$^3$, *n*=95) (Mann-Whitney test, $p<0.001$) and accounted for the 16 highest concentrations of indoor air BBzP and 88% of the highest quintile (*n*=39/44). The concentration of BBzP in the two-week air sample was correlated positively with urinary MBzP concentration (Spearman's rho 0.41, $p<0.001$). MBzP also was higher among children from homes with "vinyl or linoleum" versus other types of flooring (Mann-Whitney test,

p<0.001). There was no difference in DEHP by flooring type (p=0.93), no correlation of DEHP with its urinary metabolite mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) (rho=0.04, p=0.57), and no difference in MEHHP by flooring type (p=0.67).

Conclusions: Home materials, particularly vinyl flooring, may be an important source of exposure to BzBP for children. In addition, inhalation may be an important route of exposure to BBzP.

**Introduction**

The phthalates are a set of related compounds with widespread commercial uses (Schettler 2006). Different phthalates have different physical and chemical properties that contribute to variations in uses and patterns of human exposure. While the lower molecular weight phthalates are used in personal care products, two of the higher molecular weight phthalates, butylbenzyl phthalate (BBzP) and di(2-ethylhexyl) phthalate (DEHP) are added to plastics to give them flexibility and since they are not chemically bound, they can leach out of these materials and contaminate food, dust, and indoor air. BBzP is used primarily in the production of vinyl tiles as well as in some adhesives, carpet tiles, food conveyor belts, and artificial leather [National Toxicology Program-Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) 2003]. DEHP is primarily used as a plasticizer in flexible polyvinyl chloride (PVC) plastics and in consumer products such as flooring, car upholstery, food packaging, some toys, and medical devices (NTP-CERHR 2006). Although BBzP and DEHP have low volatility due to their high molecular weight, both are adsorbed onto respirable particles (Rakkestad et al. 2007) and considerable quantities have been found in studies collecting semi-volatile and particle bound phthalates together in personal and indoor air samples in Massachusetts, California, and New York City (Adibi et al. 2008; Rudel et al. 2003; Rudel et al. 2010).

Urinary concentrations of phthalate monoester metabolites are considered the best available biomarkers of exposure because they integrate total exposure and are not subject to contamination by plastics in sample processing and analysis (Hauser and Calafat 2005). Previous studies have found phthalate metabolites in virtually all tested samples from pregnant women, children, and in the U.S. representative NHANES (Adibi et al. 2008; Silva et al. 2004; Teitelbaum et al. 2008). While biomarker measures indicate ubiquitous exposure, concentrations of phthalate metabolites

can vary over orders of magnitude, likely due to different patterns of exposure, uptake, and/or metabolism across the population.

Controlled dosing studies in adults indicate that variability in metabolism and excretion dynamics of phthalates are substantially less than the total variability in biomarker measures, although the applicability of these results to children is unclear. In a controlled dosing study with seven adults, 73% of an ingested dose of isotope-labeled BBzP was excreted in urine as the monoester metabolite monobenzyl phthalate (MBzP) with a relative standard deviation between individuals of 39% and 26% for low and high doses (Anderson et al. 2001). In contrast to BBzP, DEHP metabolites undergo a further oxidation stage after hydrolysis to the monoester. The resulting secondary oxidative metabolites are considered better biomarkers because they are more frequently detected and have longer biologic half-lives than the monoester (Koch et al. 2006; Preau et al. 2010). In a more recent study, four DEHP metabolites were measured in urine collected over regular intervals up to 48 hours after 10 male and female adults (ages 18-77) were given labeled doses of DEHP (Anderson et al. 2011). Over 48 hours, 6% of the dose was excreted in urine as the monoester metabolite mono(2-ethylhexyl) phthalate (MEHP) with a relative standard deviation of 31%. The oxidative metabolite mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) made up the largest share with 16% of the dose and a relative standard deviation of 20%. DEHP metabolites were estimated to have a half-life of 4-8 hours with greater than 90% excretion in the first 24 hours.

In this study, we hypothesized that vinyl flooring in the home would be associated with higher indoor air concentrations of BBzP and higher urinary concentrations of MBzP among children. We also tested whether the same relationships would hold for DEHP, a ubiquitous phthalate used in PVC plastics for which children's exposure is believed to be primarily dietary

(Wormuth et al. 2006). For DEHP, we hypothesized that vinyl flooring in the home would be associated with higher indoor air concentrations but no change in the concentration of the urinary metabolite MEHHP.

## Methods

### *Cohort design and enrollment*

Participants for this study were children in the Columbia Center for Children's Environmental Health (CCCEH) longitudinal birth cohort study (Perera et al. 2003; Whyatt et al. 2003). A total of 727 women were enrolled into the cohort during pregnancy between 1998-2006 from prenatal clinics at two hospitals in Northern Manhattan. Eligible participants included non-smoking pregnant self-identified Dominican or African American women ages 18-35 who reported living within Northern Manhattan or the South Bronx for $\geq 1$ year and detailed exclusion criteria have been previously reported (Perera et al. 2003). The study was approved by the Institutional Review Board of Columbia University Medical Center and all participants signed an informed consent prior to enrollment. For this study, a subset of $n$=220 children were selected who had reached the age of 5 years and had available data from a checklist of materials that might contain phthalates in the home, and collection of an indoor air and child's spot urine sample. The spot urine was collected during indoor air sampling for $n$=176 participants. Both analytic laboratories measuring air and urine samples were blinded with regards to information on subject exposure characteristics.

*Exposure information*

Child's race/ethnicity, mother's education, and marital status were based on maternal self-report. A checklist for the presence of materials in the home that are likely to contain phthalates was administered by field staff at the time of the indoor air sampling (see Appendix to Chapter 4). The checklist was completed in the same room as the air sampling (usually the child's room) as well as several others in the home. It included observation of the type of flooring, plastic coverings on furniture, plastic blinds or window treatments, and plastic shower curtains. Flooring was categorized as one of "vinyl or linoleum", wood, carpet, ceramic tile, or other. The category of "vinyl and linoleum" flooring combined these floor types in the checklist based on prior work that these materials are not easily distinguishable by visual inspection, although phthalates are added to vinyl and not linoleum flooring products (Kolarik et al. 2008). Since there were only three homes with ceramic tile and none with "other" flooring in the room that was monitored these categories were dropped from all analyses, with $n$=220 homes remaining with wood, carpet, or "vinyl or linoleum" in the monitored room.

*Indoor air*

Two-week integrated air samples, which collected respirable particles and semi-volatiles, were used to characterize total airborne concentrations of phthalates occurring in the home environment. Stationary air monitors were set up preferentially in the child's bedroom or main living space to sample continuously at 1.5 l/min and ran for 13 to 16 days (92% of samples were 14 days). Samplers used a $PM_{2.5}$ cyclone (BGI, Waltham, MA) with a quartz filter followed by a polyurethane foam plug (PUF) encased in a modified URG personal pesticide sampler (University Research Glassware, Chapel Hill, NC). After collection, PUFs and filters were stored at -20°C

before shipment on dry ice to Southwest Research Institute in San Antonio, TX where they were extracted together before analysis with GC/MS using isotope-labeled internal standards (Adibi et al. 2008). Laboratory matrix blanks were extracted and analyzed with each batch of samples. Although there was evidence of sporadic contamination in the laboratory, the geometric mean concentration in matrix blanks (GM 39 ng BBzP per extract, 95% CI 36 to 42 ng per extract; GM 121 ng DEHP per extract, 95% CI 99 to 148 ng per extract, *n*=102) was considerably lower than among extracts from two-week indoor air samples (GM 522 ng BBzP per extract, 95% CI 452 to 603 ng per extract; GM 2706 ng DEHP per extract, 95% CI 2529 to 2896 ng per extract).

*Urine measures*

Of the 176/220 (80%) of children with spot urines collected during the two-week air sampling, 24% were collected on the first day and 48% on the last day of sampling with the remaining samples collected at clinic visits which were largely afterschool. The 44 urine samples collected outside the period of the two-week air sampling ranged from 97 days before to 47 days after air sampling. These were used primarily to explore the sensitivity of the correlation between air and urine measures to temporal concurrency. Samples were stored at -80°C until shipment on dry ice to the Centers for Disease Control and Prevention laboratory for analysis. Measurements used isotope-labeled internal standards with high performance liquid chromatography and tandem mass spectrometry (Adibi et al. 2008; Silva et al. 2007). Studies utilizing repeated samples have shown that phthalate urinary metabolite concentrations are likely stable after long-term frozen storage (Baird et al. 2010; Samandar et al. 2009), and concentrations from a single spot urine sample are reasonably representative of concentrations over several months in pregnant women in this cohort (Adibi et al. 2008). In another study of New York City children, MBzP concentrations

were more reproducible than metabolites of DEHP (Teitelbaum et al. 2008). Overall, fewer studies have analyzed exposure to phthalates in early school-aged children than adults because of the additional difficulty in enrollment and sample collection.

*Statistical analysis*

Because concentrations of air phthalates were non-normally distributed, we used non-parametric tests: Mann-Whitney tests for independent samples, and Spearman's rho for rank correlations. Concentrations of urinary metabolites were approximately log-normally distributed and were natural log transformed prior to analysis. Analyses were conducted using R version 2.13.1 with the beanplot and ggplot2 packages for graphics (Kampstra 2008; R Development Core Team 2011; Wickham 2009).

**Results**

The 220 children enrolled ranged in age from 5.0 to 10.7 years (mean 6.6 years, 90% less than 9 years) and included more Dominican than African American children (Table 1). The category "vinyl or linoleum" was the most frequently observed flooring in 57% (*n*=125/220) of the rooms monitored. Wood was the next most frequent category (32%, *n*=71/220), followed by carpet (11%, *n*=24/220). There was no association between flooring type and mother's race/ethnicity, mother having ever married, or mother having completed high school.

**Table 1.** Characteristics of (*n*=220) children and mothers

| | |
|---|---|
| Child's Age (years) mean (SD) | 6.6 (1.7) |
| Child's Sex (% male) | 48 |
| Ethnicity<br>African American (%) | 33 |
| Dominican (%) | 67 |
| Mother's Education<br>High school or greater (%) | 69 |
| Mother's Marital Status<br>Never married (%) | 65 |

Missing: mother's marital status (*n*=1)

Phthalates were detected in all air samples and urinary metabolites were detected in all urine samples. Distributional summary statistics for the concentrations of phthalates in two-week indoor air samples and urinary metabolites from children's spot urines are shown in Table 2. The concentrations of BBzP in indoor air were overall lower than those of DEHP ($p<0.001$). There was no difference in metabolite concentrations in urine samples collected during versus not during the air sampling (not shown).

**Table 2.** Distributional statistics for concentrations of indoor air phthalates and urinary metabolites (*n*=220)

| | | | Percentile | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Phthalate diester | Phthalate metabolite | Percent > LOD[a] | Min | 25% | 50% | 75% | 95% | Max | GM (95% CI) |
| ($ng/m^3$) | | | | | | | | | |
| BBzP | | 100% | 2 | 8 | 13 | 32 | 186 | 353 | 17 (15 to 20) |
| DEHP | | 100% | 28 | 61 | 82 | 122 | 241 | 353 | 89 (83 to 95) |
| | (ng/ml) | | | | | | | | |
| | MBzP | 100% | 1 | 17 | 35 | 76 | 235 | 1950 | 36 (31 to 42) |
| | MEHP | 90% | <LOD | 2 | 5 | 11 | 39 | 333 | 5 (4 to 6) |
| | MEHHP | 100% | 2 | 24 | 54 | 115 | 326 | 1690 | 52 (45 to 61) |

LOD, limit of detection; GM, geometric mean

[a]*n*=37 air samples were below the lowest standard for BBzP leading to imprecise quantitation; *n*=21 samples were below the LOD for MEHP, which was in the low ng/ml range, and these concentrations were imputed with a value of ½ the LOD

As can be seen in Figure 1, there was no significant difference between BBzP concentrations measured in rooms with carpet versus wood flooring (p=0.27). Rooms with "vinyl or linoleum" flooring had a significantly higher concentration of BBzP (GM 24.3; IQR [9.3, 66.8] ng/m$^3$), than homes with wood or carpet flooring (GM 10.9; IQR [6.9, 14.1] ng/m$^3$, *n*=95) (p<0.001). The homes with "vinyl or linoleum" flooring in the room being monitored accounted for the 16 highest concentrations of indoor air BBzP and 88% of the highest quintile (*n*=39/44). However, they also spanned the range of the exposure distribution for the three types of flooring (2.4 to 353 ng/m$^3$ BBzP). As shown in Figure 1, there was no difference in the distribution of indoor air DEHP by flooring type (p=0.93). A separate question that characterized the total proportion of the flooring of the home that was "vinyl or linoleum" found marginal associations for a higher distribution of BBzP in homes with more than half of the home having "vinyl or linoleum" flooring after stratifying by the flooring in the room being monitored (Figure 2). Further, the flooring in the monitored room was associated with the categorization of the total flooring in the home. There were only seven homes with carpet or wood in the monitored room that had greater than 50% total "vinyl or linoleum" flooring and only ten homes with "vinyl or linoleum" flooring in the monitored room that had less than 50% total "vinyl or linoleum" flooring.

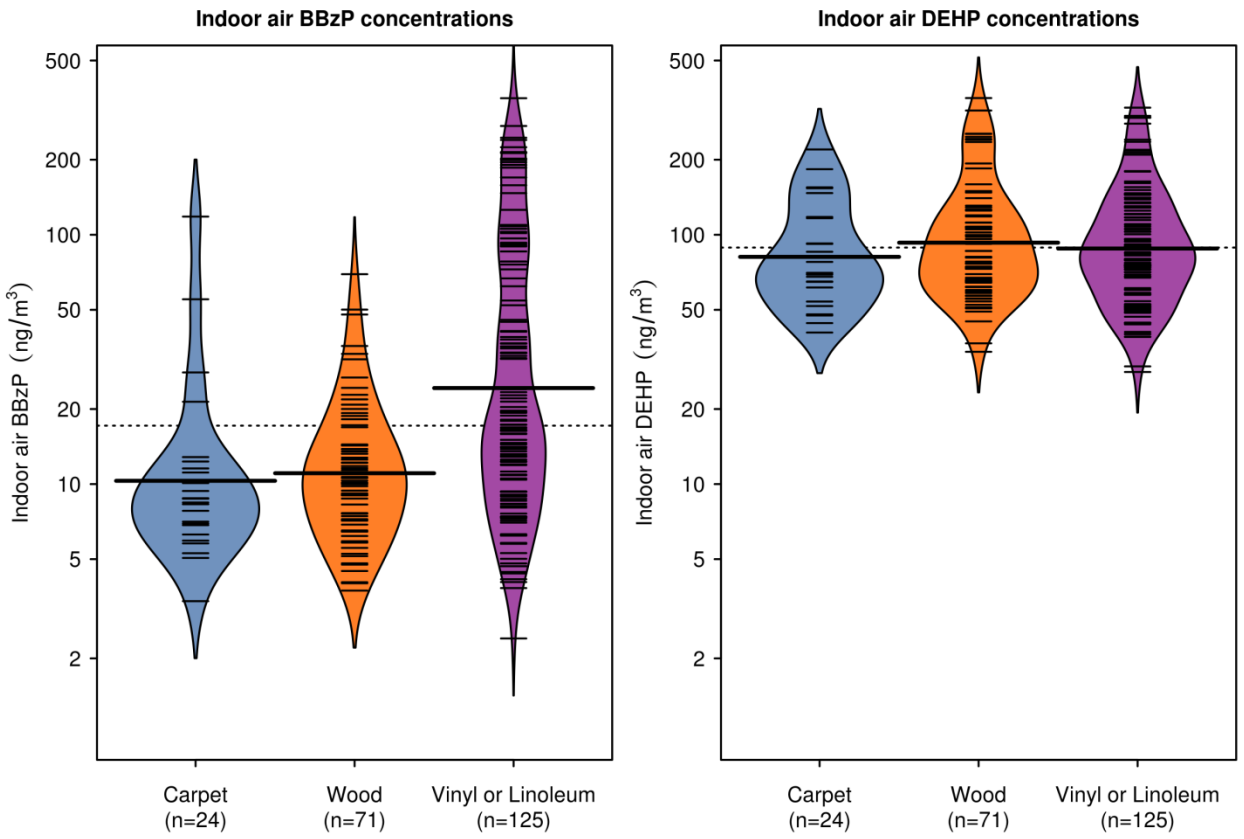


**Figure 1: Beanplots show the distribution of BBzP and DEHP concentrations in two-week indoor air samples by type of floor in the room being monitored (*n*=220).**
There was no difference in BBzP between carpet and wood (p=0.27) although those two groups together were substantially lower than "vinyl or linoleum" (p<0.001). The 16 highest values were in the "vinyl or linoleum category", which made up 39/44 homes in the top quintile greater than 40 ng/m$^3$. There was no difference between "vinyl or linoleum" and the combination of carpet and wood for DEHP (p=0.93). Each horizontal dash is one home with group geometric means shown as darker black lines and dotted grand geometric means for each phthalate. The shape of the 'bean' gives the weight of the distribution from a kernel density estimate.

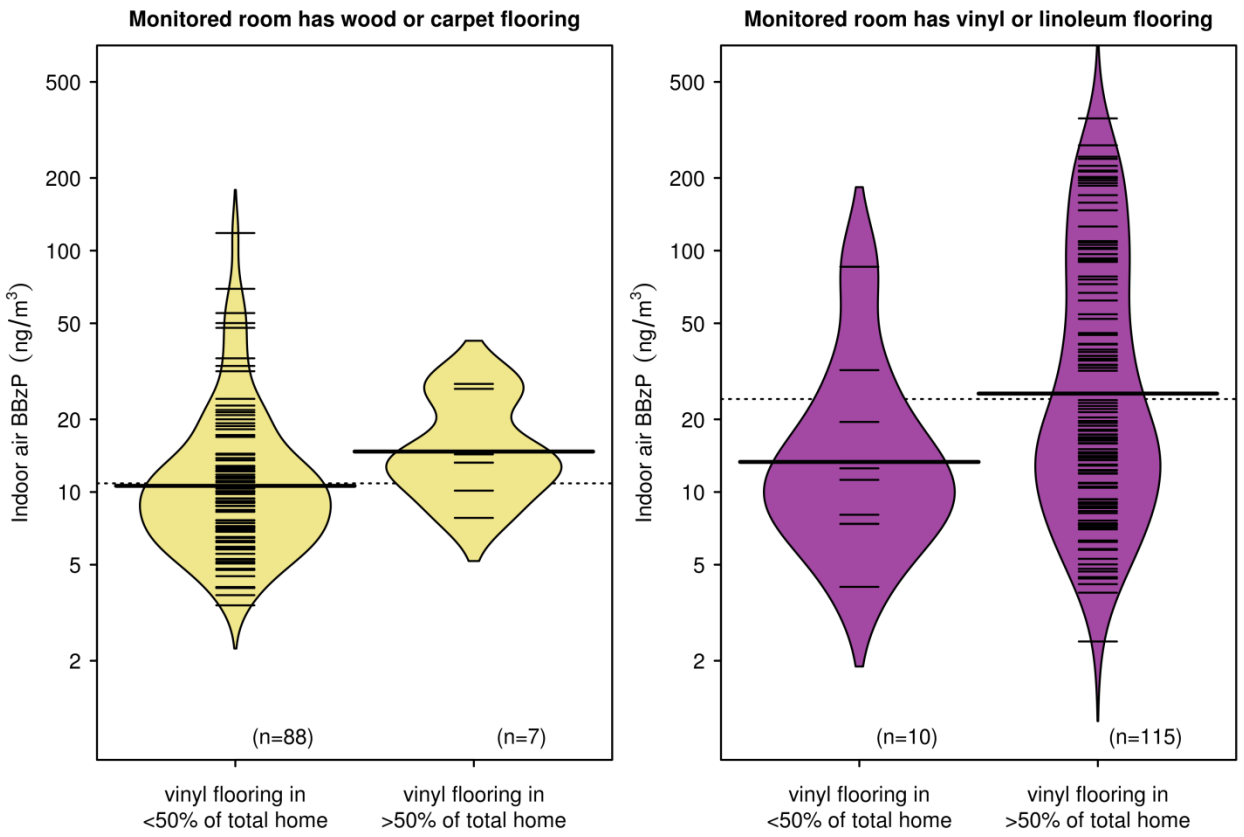


**Figure 2: Distribution of concentrations of BBzP in two-week indoor air samples and whether half or more of the total home has "vinyl or linoleum" flooring, stratified by floor type in the room with the monitor.**
The small sample sizes in the discordant cells indicate that the flooring in the room being monitored was representative of the home. Nonetheless, these associations were suggestive that the flooring in the remainder of the home contributed to the indoor air concentration measured in a single room (p=0.07 and p=0.09).

Indoor air concentrations of BBzP and DEHP were correlated in homes with carpet or wood flooring in the monitored room (rho=0.33, p=0.001, *n*=95), but not in homes with "vinyl or linoleum" flooring (rho=0.03, p=0.72, *n*=125) (Figure 3). Among the homes with "vinyl or linoleum" flooring, a subset had indoor air concentrations of BBzP that exceeded the indoor air concentration of DEHP (20%, *n*=25/125).

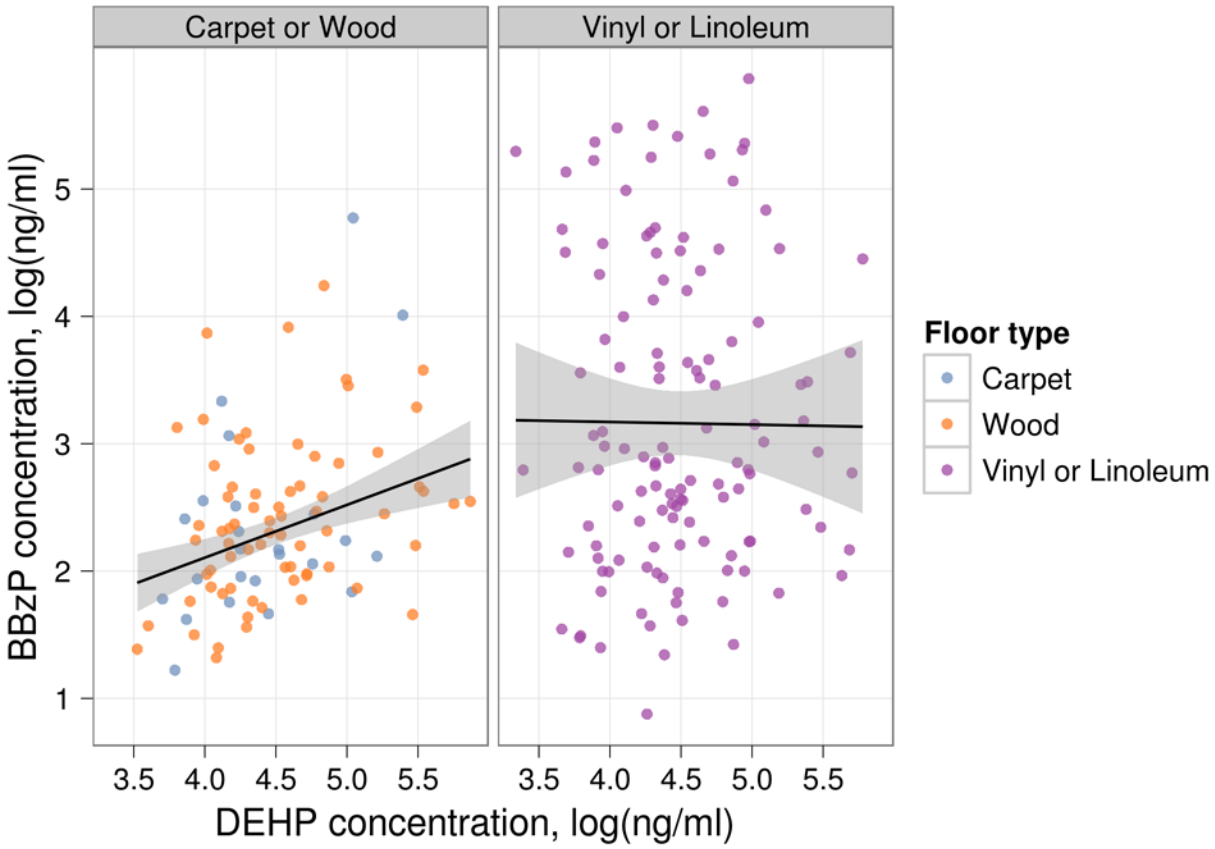


**Figure 3: Association between indoor air concentrations of DEHP and BBzP stratified by floor type.**
There is a correlation between indoor air DEHP and BBzP concentration only among those with carpet or wood (rho = 0.33, p = 0.001, *n* = 95) and not vinyl or linoleum flooring (rho = 0.03, p = 0.72, *n* = 125) in the room monitored. Lines show robust linear models with shaded 95% confidence intervals.

The concentration of BBzP in the two-week air sample was correlated positively with concurrent urinary MBzP concentration (rho=0.41, p<0.001, *n*=176); a similar correlation was seen in a subset of urine samples collected five or more days before or after the air sampling (*n*=20, median 13 days apart, rho=0.37, p=0.11). The concentration of DEHP was not correlated with concurrent MEHP (rho=0.02, p=0.75), or MEHHP (rho=0.04, p=0.57).

The distributions of urinary metabolite concentrations by floor type are shown in Figure 4. Urinary MBzP concentrations were also higher among children with “vinyl or linoleum” in the monitored room versus the combination of carpet or wood (p<0.001). As in the air monitoring,

there was no difference in the distribution of urinary MBzP concentration between the carpet and wood (p=0.83). There was no difference in the distribution of urinary MEHHP concentrations between children with “vinyl or linoleum” in the room monitored versus the combination of carpet and wood (p=0.67).

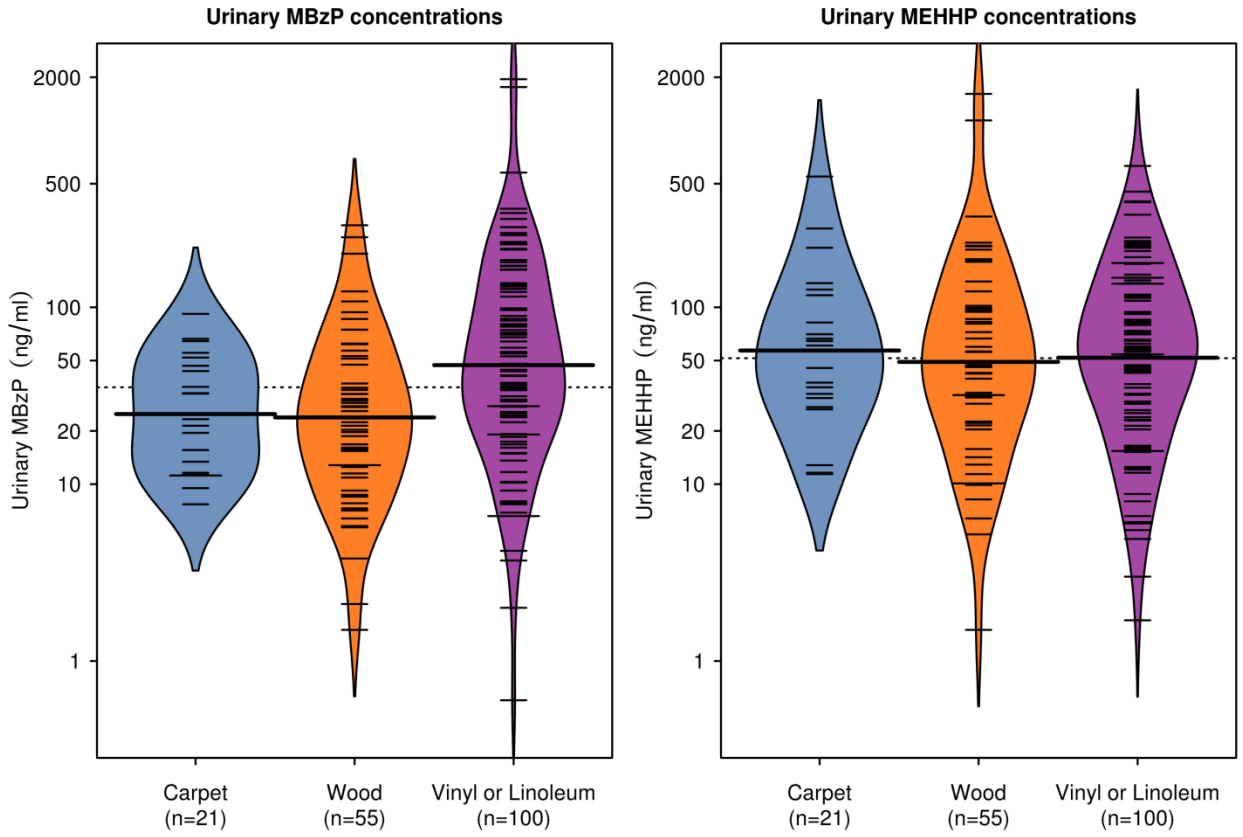


**Figure 4. Beanplots show the distribution of urinary concentrations of MBzP and MEHHP in spot urine collected during the air sampling by type of floor in the room being monitored (*n*=176).**

There was no difference in MBzP concentration between carpet and wood (p=0.83), and these two categories together was significantly lower than “vinyl or linoleum” (p<0.001). There was no difference between “vinyl or linoleum” and the other two categories for MEHHP (p=0.67). Each horizontal dash is one home with group geometric means shown as darker black lines and dotted grand geometric means for each phthalate. The shape of the ‘bean’ gives the weight of the distribution from a kernel density estimate.

**Discussion**

This study is consistent with previous findings in both Sweden and Bulgaria that vinyl flooring is an important predictor of concentrations of BBzP in home air and dust (Bergh et al. 2011; Bornehag et al. 2005; Kolarik et al. 2008). The addition of biomonitoring data in this study also demonstrates that home flooring may be an important source of internal exposure to BBzP among children. Further, the correlation in the current study between BBzP and MBzP concentrations in air and urine supports previous findings that the indoor environment may be an important source of exposure for this phthalate via inhalation, dermal absorption, or incidental ingestion. The correlations seen here were similar but somewhat lower than seen previously in a study that measured BBzP in maternal 48-hour personal air and spot urine samples (rho=0.48, $p<0.05$, $n$=62) (Adibi et al. 2008). This lower correlation may be due to a larger mismatch in the duration of the air monitoring versus the short biologic half-life of hours for the urinary metabolite, or to the contribution of BBzP in air from other micro-environments outside the home that was captured by backpack monitoring in the previous study. However, repeated sampling of both BBzP in two-week indoor air and urinary MBzP in maternal spot urines have previously shown good reproducibility in 2-4 samples collected over 6-8 weeks (ICC 0.83, n=32 for BBzP; ICC 0.66, $n$=28 for MBzP). The lack of a correlation between concentrations of DEHP in the indoor air samples and metabolites in urine samples is also consistent with the prior study of personal air and urine concentrations seen in the mothers in the current cohort (Adibi et al. 2008), as well as a German study measuring DEHP in sieved vacuum cleaner bag dust and spot urine samples in $n$=254 children aged 3 to 14 (Becker et al. 2004). Consistent with these results, a European exposure model using scenario-based probabilistic source apportionment calculated that indoor air contributes 26% of children's exposure to BBzP with diet contributing 73% of exposure. By

contrast, diet was estimated to contribute >90% of children's exposure to DEHP, with inhalation from indoor air making up the remainder (Wormuth et al. 2006).

Although these results support vinyl flooring as an important source of BBzP, substantial concentrations were still measured in the indoor air of homes with no observable vinyl flooring. We speculate that shared sources of both BBzP and DEHP such as consumer products containing plasticizers, or else a shared reservoir in house dust may explain the correlation of BBzP and DEHP concentrations in homes without vinyl flooring.

We also observed considerable variability in the indoor air concentrations of BBzP among homes with "vinyl or linoleum", in the room that was monitored. Some of this may be attributable to homes using linoleum flooring, which would contain no plasticizers. The distribution of BBzP concentrations among homes with "vinyl or linoleum" flooring remained skewed after log transformation and had an appearance that would be consistent with a mixture of a log-normal distribution similar to those in the carpet or wood floor homes and a second distribution that was higher and more variable. There was also no information available on the composition, brand, age, or condition of the flooring, particularly the presence of water damage, which are likely to be important parameters in explaining the contribution of flooring to indoor air phthalate concentrations. Most of the homes monitored in this study had little variability in flooring type within the major rooms of the home. Although the category of "vinyl or linoleum" flooring was quite common in this population, we do not know if the types of materials used in housing in New York City would be representative of those in other areas.

In this study, the presence of particular flooring materials, a combination of vinyl or linoleum, was a sensitive but non-specific factor accounting for nearly every home in the highest range of indoor air concentration of BBzP. The correlation of BBzP and urinary MBzP, and the

association of flooring with metabolite concentrations further supports the indoor environment as a substantial and chronic contributor to children's exposure to the phthalate plasticizer BBzP.

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## Appendix: home materials checklist

### Home Materials Checklist for Tapas Phthalate Monitoring

SID: _____ visiting at: IN – 1 / IN – 2 / IN – 3 DATE:__ __ / __ __ / __ __ __ __ SUBJ INITIALS:_____

**Room 1** with air sampling

**1a. Location:**

living room.....1
child's room.....2
parent's room.....3
other (specify).....8

**1b. Flooring**

Wood.....1
Vinyl or Linoleum.....2
Ceramic Tile.....3
Carpet.....4
NA.....7
Other (specify).....8

**1c. Window Treatments** Vinyl or plastic curtain, liner, or blinds

Yes.....1
No.....2
NA.....7

**1d. Furniture Covers** Any furniture with plastic covering?

Yes.....1
No.....2
NA.....7

**1b1. If NOT carpet:** partial rug or throw rugs (approximately >25%)?

Yes.....1
No.....2

**1e. Does the child sleep in the room with the air monitor?**

Yes.....1
No.....2

**If YES skip Room 2**

**Room 2** where child sleeps (if different room from above)

**2b. Flooring**

Wood.....1
Vinyl or Linoleum.....2
Ceramic Tile.....3
Carpet.....4
NA.....7
Other (specify).....8

**2c. Window Treatments** Vinyl or plastic curtain, liner, or blinds

Yes.....1
No.....2
NA.....7

**2d. Furniture Covers** Any furniture with plastic covering?

Yes.....1
No.....2
NA.....7

**2b1. If NOT carpet:** partial rug or throw rugs (approximately >25%)?

Yes.....1
No.....2

**Room 3** next largest room

**3a. Location:**

living room.....1
other bedroom.....2
parent's room.....3
dining room.....4
kitchen.....5
NA.....7
other (specify).....8

**3b. Flooring**

Wood.....1
Vinyl or Linoleum.....2
Ceramic Tile.....3
Carpet.....4
NA.....7
Other (specify).....8

**3c. Window Treatments** Vinyl or plastic curtain, liner, or blinds

Yes.....1
No.....2
NA.....7

**3d. Furniture Covers** Any furniture with plastic covering?

Yes.....1
No.....2
NA.....7

**3b1. If NOT carpet:** partial rug or throw rugs (approximately >25%)?

Yes.....1
No.....2

**Bathroom which child uses most:**

**4a. Flooring**

Wood.....1
Vinyl or Linoleum.....2
Ceramic Tile.....3
Carpet.....4
NA.....7
Other (specify).....8

**4b. Shower curtain/door** Vinyl/Plastic Curtain or Liner?

Yes.....1
No.....2
NA.....7

**6a. Do any other rooms have vinyl or linoleum flooring?**

Yes.....1
No.....2
NA.....7

**6b. Estimate the proportion of the total household flooring that is vinyl or linoleum:**

<25%.....1
25% to 50%.....2
50% to 75%.....3
>75%.....4

revision date: 1/23/2007
*NA is to be used to indicate missing data such as when you cannot get into the room*

Technician Initials:_____

# Chapter 5: Bayesian models for correlated biomarkers of exposure: multiple phthalate metabolites and association with gestational age

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**Abstract**

**Background:** Our prior analyses showed that concentrations of four metabolites of di(2-ethylhexyl) phthalate (DEHP) measured in prenatal spot urines were individually and as a molar sum associated with shortened gestation in an inner-city pregnancy cohort. The high multicollinearity of the phthalate metabolites poses a challenge in modeling multiple exposures simultaneously. Bayesian methods, which can stabilize estimation and incorporate prior knowledge have not been widely applied to biomonitoring data. The current study is the first application of Bayesian regression models to assess associations between highly correlated biomarkers and a continuous outcome.

**Methods:** We compare models that fit one phthalate metabolite at a time with a full multiple linear model and with the following Bayesian models: model 1 equivalent to ridge regression, and model 2 a simple hierarchical Bayes model that groups regression coefficients of similar predictors. We assess whether Bayesian methods improve parameter estimation of the association between urinary concentrations of multiple phthalate metabolites and gestational age. Sensitivity analysis was performed to allow various prior distributions of exposure effects.

**Results:** Parameter estimates from Bayesian models are more stable with smaller variance estimates compared with those from the multiple linear model for highly correlated exposures. No single phthalate metabolite is significantly associated with gestational age, although three have relatively large negative effect estimates: mono(3-carboxypropyl) phthalate, a metabolite of high molecular weight phthalates including di-*n*-octyl phthalate and two DEHP metabolites, mono(2-ethylhexyl) phthalate and mono(2-ethyl-5-hydroxyhexyl) phthalate.

**Conclusions: Several phthalate metabolites appear together to be associated with reduced gestational age but no single metabolite predominates.**

## Introduction

Phthalates are plasticizers commonly used in household and consumer products to confer flexibility (Schettler 2006). Exposure to mixtures is common with metabolites of a number of phthalate metabolites detected in 100% of urine samples (Adibi et al. 2008). These concentrations are often highly correlated both because some metabolites share common parent compounds and because phthalates can have similar sources of exposure. Preliminary epidemiology has shown associations between a number of phthalates and male urogenital development, neurodevelopment, asthma, and modulation of the length of gestational age (Bornehag and Nanberg 2010; Engel et al. 2009; Swan 2008; Whyatt et al. 2009). Thus, the phthalates (and their metabolites) represent one example of a common mixture of environmental contaminants for which appropriate epidemiologic models are needed in order to estimate the association with health outcomes of interest, a recommendation of a recent report on cumulative assessment of the phthalates by the National Academies (Committee on the Health Risks of Phthalates 2008).

Several data analytic techniques have been used previously for estimating associations of concentrations of multiple phthalate metabolites with health outcomes. These include conventional strategies for handling multiple (correlated) variables in regression analyses: analyzing one metabolite concentration at a time using multiple models (Whyatt et al. 2009), pretesting for statistical significance in order to select subsets of phthalates (Swan et al. 2005), and construction of summary scores with fixed component weightings (Engel et al. 2009). Because exposures occur simultaneously, models that estimate associations of components of mixtures with health outcomes one at a time as though each were the only relevant environmental predictor fail to take into account potential confounding effects from other environmental exposures and can lead to biased effect estimation. By contrast, ordinary least squares (OLS) estimates of regression coefficients in a

multiple linear model could be unstable with all the highly correlated environmental exposures in the same model. This phenomenon is called multicollinearity in a multiple regression. A more desirable statistical technique, such as a Bayesian regression model, would take into account all of the relevant exposures and offer insight into the relative importance of the mixture components (Greenland 1994). Bayesian regression models extend multiple linear regression models by treating the exposure effects as random variables, which can depend on second level covariates through some prespecified distributions. The principal purpose of the current study was to compare four statistical modeling approaches for highly correlated biomarkers: two regular linear regression models and two Bayesian models. Our goal is to provide some regression techniques that produce stable and reasonable regression estimates of the association of multiple correlated exposure biomarkers with a continuous health outcome.

*Bayesian Models*

We consider the following Bayesian regression model without intercept, in which the continuous outcome variable, $y_i$, is centered at its mean:

$$y_i \mid \beta_j \sim N\left(\sum_{j=1}^{p} \beta_j x_{ij}, \sigma^2\right)$$

$$\beta_j \sim N\left(\mu, \tau^2\right). \qquad (1)$$

Where, $\beta_1, \cdots, \beta_p$ are effect measures for exposures $x_1, \cdots, x_p$. In contrast to estimates from a linear regression model fit using OLS, in model (1) the parameter estimates $\beta_1, \cdots, \beta_p$ are treated as random and follow a prior normal distribution with mean, $\mu$, and variance $\tau^2$. The estimates of $\beta_1, \cdots, \beta_p$ obtained though model (1) are moved away from the OLS estimates and toward $\mu$, the center of the prior distribution. This procedure, which adds a small amount of bias to parameter

estimates but reduces variance estimates, is known as shrinkage. This bias-variance tradeoff often results in estimates with smaller mean squared errors (the combined effect of bias and variance) than the OLS estimates, especially in the presence of multicollinearity.

When $\mu = 0$, Model (1) is equivalent to a ridge regression model with a penalty term for shrinkage, where the shrinkage parameter $\lambda = \sigma^2 / \tau^2$ . Ridge regression is one of several methods that have been proposed to remedy the problem of predictor multicollinearity (Hastie et al. 2009). Ridge regression is fit by statistical packages via matrix operations as a penalized regression model (Venables and Ripley 2002). The shrinkage parameter $\lambda$ determines how much the regression estimates move away from the maximum likelihood estimate and toward zero. Specifically, when $\lambda = 0$, it is equivalent to an ordinary linear regression; and when $\lambda$ = infinity, it is an intercept only model with all regression coefficients forced to 0. The shrinkage parameter can be chosen so as to minimize the estimated prediction errors via generalized cross-validation (Golub et al. 1979; Hastie et al. 2009).

An important assumption in the ridge equivalent Bayesian model is exchangeability of the exposure effects which are put under a common prior distribution, meaning that the data analyst believes that the magnitude of associations from various predictors likely come from the same prespecified probability distribution. If previous evidence exists that predictors can be categorized into groups, where the effects of predictors are more similar within groups than between groups, the Bayesian model can be restructured to incorporate this grouping (Momoli et al. 2010). This grouping can be achieved by allowing $\beta$ in model (1) to depend on the effects of a group indicator and its prior mean. Model (1) can be rewritten as,

$$y_i \mid \beta_j \sim N\left(\sum_{j=1}^{p} \beta_j x_{ij}, \sigma^2\right),$$

$$\beta_j \sim N\left(\sum_{g=1}^{G}\gamma_g z_{gj}, \tau^2\right),\ \gamma_g \sim N\left(\mu_g, \phi_g^2\right),$$

$$\tau^2 \sim IG\left(\alpha_1, \alpha_2\right),\ \sigma \sim Uniform(0,10^3), \qquad (2)$$

where exposures are clustered into G groups and $z_{gj}$ is group indicator for the $j^{th}$ exposure. The effect of belonging to the $g^{th}$ group is $\gamma_g$, whose prior mean, $\mu_g$, is the prior knowledge regarding the size of $\gamma_g$, and the prior variance, $\phi_g^2$, represents uncertainty about that effect size. Unlike the fixed shrinkage in model (1), model (2) allows for adaptive shrinkage and makes estimates more data driven (MacLehose et al. 2007). This grouped hierarchical Bayes model (2) also relaxes the global exchangeability of the exposure effects and thus improves model fit and reduces mean squared errors. Annotated statistical code for use with R and WinBUGS to allow investigators to run model (1) and model (2) on other datasets is included in Appendix 3.

**Methods**

*Study design*

As explained in detail elsewhere (Whyatt et al. 2009), participants were pregnant African American and Dominican women with prenatal urine specimens available for analysis and medical record data from the Columbia Center for Children's Environmental Health study of Mothers and Newborns (Perera et al. 2003). All participants signed consent, approved by the IRB of Columbia University, at the time of study enrollment. Our selection criteria of study subjects were similar to our prior analyses of the association between prenatal concentrations of DEHP metabolites and gestational age, with the following modifications. In our previous analysis we controlled for the subsequent report of active smoking during pregnancy, however, here we excluded 10 such individuals because active smoking was an exclusion criterion at enrollment for the cohort. Due to

the selection of a smaller set of other covariates not previously shown to act as confounders (Whyatt et al. 2009), the sample size of participants with complete data was 249, of these 233 were also in the fully adjusted model of our prior analysis.

*Phthalate measurement*

A spot urine was collected during the third trimester of pregnancy. Specific gravity was measured with a handheld refractometer at room temperature, and a total of eight urinary phthalate metabolites, listed in Table 1 with their parent compounds, were measured at the Centers for Disease Control and Prevention (Kato et al. 2005). Details on the prenatal exposure to phthalates in this cohort have been published elsewhere (Adibi et al. 2008; Just et al. 2010; Whyatt et al. 2009). Several phthalate metabolites whose concentrations were not available for the entire cohort (monomethyl phthalate, monocarboxy-isooctyl phthlate, and monocarboxy-isononyl phthlate) were excluded from further analysis. Samples with concentrations that were below the limits of detection, which were in the low nanogram per milliliter range for all metabolites, were imputed with a value equal to ½ the limit of detection.

*Gestational age and predictors of interest*

Gestational age at delivery, measured in weeks, was extracted from medical records (Whyatt et al. 2009). In our previous analysis, we examined many potential confounders and reported adjusted effect size estimates primarily to increase model precision rather than because of evidence of confounding (Whyatt et al. 2009). In the current analysis, we included two potential confounders that were previously statistically significant predictors of gestational age in this dataset, planned Cesarean section, and premature rupture of membranes, which were both yes/no

variables. However, it should be noted that our previously reported associations between urinary concentrations of DEHP metabolites and gestational age were unchanged in the reduced linear regression models with this slightly different set of participants (Whyatt et al. 2009).

Because of the extreme correlation between the urinary concentrations of two of the secondary oxidative metabolites of DEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP) ($r = 0.98$) which is presented below in results, and because MEHHP concentrations had a stronger association with gestational age than MEOHP concentrations when tested individually, we removed MEOHP concentrations from our regression modeling.

Urinary metabolite concentrations were adjusted for dilution relative to the median specific gravity among this cohort of pregnant women, a modification of the method of Hauser and colleagues (Hauser et al. 2004; Just et al. 2010). Phthalate metabolite concentrations were subsequently log transformed as their concentrations, like many other exposure biomarkers, are right skewed. As shrinkage estimation techniques are not scale invariant, we standardized our regression predictors, including both continuous and binary variables, by subtracting the mean and dividing by the standard deviation to put them all on a common scale of standard deviation units.

*Modeling strategies*

We used penalized splines to check for non-linear associations between individual phthalate metabolites and gestational age (details in Appendix 1). Since our results showed an inverse association consistent with linearity, we used our predictors as linear terms in our regression models. We report the results from four modeling approaches using an expanded set of phthalate metabolites relative to our previous analysis. All of the models were fit without an

intercept using a centered outcome by subtracting the mean gestational age from each record before regression (mean 39.3 weeks, range 34 to 42 weeks). The two conventional model fitting strategies were to model each of the eight metabolites concentrations one at a time ("Single Models") and to model the effects of all metabolites concentrations together in a multiple linear regression model using OLS ("Full OLS"). Both model strategies adjusted for the effects of the other two covariates: planned Cesarean section and premature rupture of membranes.

In addition, we fit two Bayesian models. The first was a simple Bayesian model equivalent to ridge regression on the multiple regression model ("Bayes-Ridge"). The second Bayesian model as shown in Model (2), also fit with the full set of predictors, added some flexibility by allowing the prior means of the exposure effects to vary in different groups of similar predictors ("Bayes-Group"). The selection of predictor variables into groups in the second Bayesian model was based on the belief that concentrations of DEHP metabolites were more likely to have similar effects sizes than the metabolites of other phthalates or the other (non-phthalate) covariates. The three groups were non-DEHP metabolites (5 predictors), DEHP metabolites (3 predictors), and other covariates (2 predictors). Prior means of effect measures were centered at zero, and prior variances were selected to correspond to the penalization term that minimized prediction error in ridge regression based on a generalized cross-validation statistic. This selection of the prior parameters and a sensitivity analysis using different values for this prior variance are both discussed further in our Appendix 2.

R (version 2.12.2) and WinBUGS (version 1.4.3) were the primary statistical systems used (Lunn et al. 2000; R Development Core Team 2011). The R package "R2WinBUGS" was used to call WinBUGS and export results into R (Sturtz et al. 2005). Graphics were created in R using the "ggplot2" package (Wickham 2009).

**Results**

The distributions of phthalate metabolite concentrations (before adjustment with specific gravity) are presented in Table 2. The correlations between concentrations of phthalate metabolites were all positive and ranged from 0.07 to 0.98 and are shown in Table 3. Concentrations of metabolites from the same parent compound (such as MEHP and MEHHP, r = 0.84) are more highly correlated than metabolites from different parent compounds (such as MiBP and MBzP, r = 0.41). All of the scatterplots that show patterns of correlation appear to have a linear relation after logarithmic transformations.

We used four different modeling strategies with the concentrations of eight target phthalate metabolites and the two other covariates (premature rupture of membranes and planned Cesarean section). The resulting coefficient estimates for standardized predictors from these four modeling strategies are shown in Figure 1.

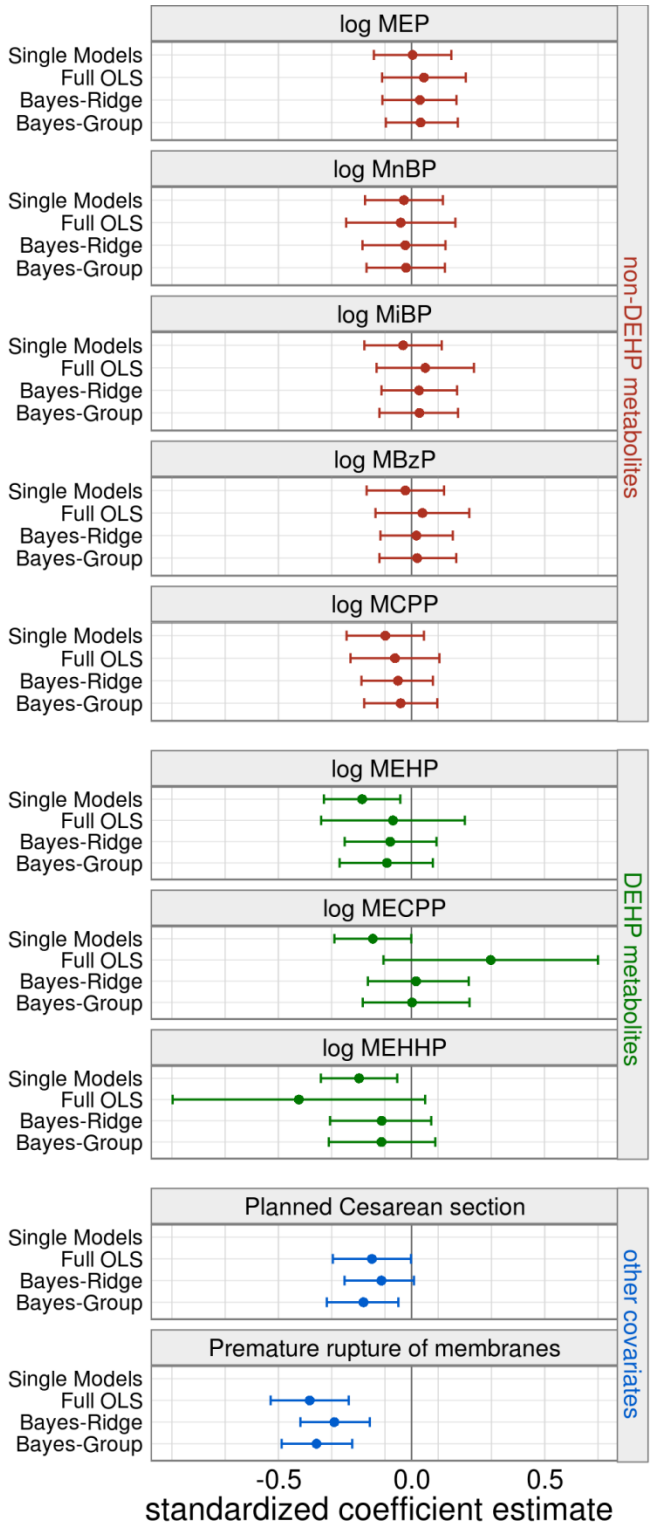


**Figure 1. Comparison of coefficient estimates and 95% confidence/credible intervals from four modeling strategies.**

Single models (one phthalate + 2 covariates in each model), a full model with all predictors fit with ordinary least squares linear regression (Full-OLS), a Bayes–Ridge full model, and a Bayes–Groups full model with grouping of similar predictors. Magnitude represents change for a one

standard deviation change in predictor on the mean gestational age in an urban cohort (n = 249). Phthalate metabolite concentrations are adjusted for specific gravity and log transformed prior to standardization. Because the two other covariates were included in each of the 8 single models, their many estimates are not shown.

Parameter estimates for non-DEHP metabolites were close to zero in all models, except for MCPP although the 95% credible interval for this parameter estimate still spanned no effect in both Bayesian models.

In single models, concentrations of the three DEHP metabolites are negatively associated with gestational age with very similar parameter estimates (-0.19, -0.14, and -0.20 change in weeks of gestational age for a one standard deviation change in log adjusted metabolite concentration for MEHP, MECPP, and MEHHP respectively). However, the parameter estimates for these metabolites were highly unstable in the Full OLS model. This is best demonstrated by the large and opposite estimates and wide 95% confidence intervals for MECPP and MEHHP, despite their pairwise correlation being as high as 0.93. By contrast, DEHP metabolites in both Bayes models have more reasonable parameter estimates with smaller variances. The sum of the parameter estimates from the three DEHP metabolites (MEHP + MECPP + MEHHP) in the Bayes models (-0.17 for Bayes-Ridge and -0.20 for Bayes-Group) is similar to the range of parameter estimates for each of these three DEHP metabolite in the corresponding single models. Both Bayesian modeling strategies indicate that MCPP, MEHP, and MEHHP might be most important because they have the largest (negative) magnitude in their parameter estimates but none of these individually achieve statistical significance.

Both the Bayes-Ridge and Bayes-Group models reduce the variance with more narrow 95% CIs for phthalate parameter estimates compared with the conventionally fit Full OLS model. In addition, in the Bayes-Group model the estimates for the two other covariates, premature rupture of the membranes and planned Cesarean section, retain larger magnitudes (more negative)

compared with the Bayes-Ridge model and are more similar to their estimates in the Full OLS model. It is worth emphasizing that these two other covariates are quite different in interpretation and distributional properties from the phthalate exposures. Although their inclusion in the model did not substantially alter effect size estimates making them not appear to be confounders by criteria of collapsibility (data not shown), they were better predictors than the phthalate metabolites. A linear regression model with premature rupture of the membranes and planned Cesarean section had an adjusted $R^2$ of 0.10 while the addition of the single metabolite with the strongest association, MEHHP, only increased the adjusted $R^2$ to 0.12.

A sensitivity analysis that used different values for the prior variance (multiplying by ½ or by 2) as well as modifying the structure of the data level and group-level error terms did not substantially vary our findings. Further discussion can be found in our Appendix 2.

**Discussion**

Consistent with our prior analysis, we observe a linear decrease in gestational age associated with urinary concentrations of metabolites of DEHP modeled one at a time. However, our analysis suggests that results from a series of single models can be misleading when predictors are highly correlated. We used three groups of predictors: five non-DEHP metabolites concentrations, three DEHP metabolites concentrations, and two other covariates. The concentrations of non-DEHP metabolites are correlated but not as highly as the DEHP metabolites that share a common parent compound. Because most of the non-DEHP metabolites concentrations have small estimated effect sizes and the correlations among them are modest, the results across models within this group are relatively similar. It may be notable that the largest effect size in this

group is for MCPP which, while not significant, comes from a metabolite of a larger molecular weight phthalate more similar in structure to DEHP than the other members of this group.

Since concentrations of the DEHP metabolites, MEHP, MECPP, and MEHHP, are highly correlated, the magnitude of their regression coefficients in the single models is similar and is notably close to the sum of regression coefficients associated with these three metabolites in the Bayesian models. Therefore, when we interpret the regression coefficient in the single models for MEHP, MECPP, and MEHHP, we should be cautious that the regression coefficient cannot be interpreted as the effect of a single metabolite only. It may represent the overall effect due to a set of highly correlated metabolites.

The concentrations of DEHP metabolites are all highly correlated. Indeed, because MEHHP and MEOHP are so highly correlated ($r = 0.98$), we decided to exclude the latter from our regression analyses. Even after this analytic decision, models that include all three of the remaining DEHP metabolites exhibit instability, which inflates our problems in parameter estimation in our Full OLS model. Since the effects of MEHHP and MEOHP are indistinguishable due to their almost perfect correlation, we should be cautious when interpreting the regression coefficient associated with MEHHP in a model excluding MEOHP. This coefficient should be interpreted as the mean effect on gestational age with a one standard deviation increase in both log-MEHHP and log-MEOHP.

Both Bayesian models stabilize the phthalate parameter estimates by pulling them towards the prior mean, but the Bayes-Group model also allows the two other covariates to retain larger magnitudes that are more similar to their magnitude in the Full OLS model. This may indicate that the more flexible grouped model makes more reasonable assumptions by relaxing the exchangeability of the other covariates with the phthalate metabolites. Adding grouping by

assigning predictors into classes of similar exposure variables can improve estimation and prediction (model fit) where assumptions of exchangeability of all of the predictors may not be warranted. Grouping similar classes of variables represents the incorporation of outside information. The use of indicators for groups allows sets of parameter estimates to be pulled towards separate means, although if additional information were available this could similarly be used in this second level of the model. For example, data on the toxicity of individual predictors or their constituents which mediate the association between measured predictors and the outcome could be used as second level covariates (Witte et al. 1994).

Previous epidemiologic studies of the phthalates have used two strategies for constructing summary variables for the highly correlated predictors in addition to the previously discussed single models. The first approach involves the construction of molar sums for high and low molecular weight phthalates (summing metabolites > and < 250 Da respectively) (Wolff et al. 2008). This approach imposes an assumption of molecular equivalence within each class, which is a strong assumption in the absence of relevant toxicology data. In addition, the molar sum measures can be strongly influenced by the highest concentrations, often MEP and the DEHP metabolites for low and high molecular weight phthalates, respectively, which may drown out potential true associations with other metabolites. These combination metrics may also be less comparable across studies than results from full models, which may measure different sets of predictors or where the relative exposure to different contaminants varies substantially such as between studies conducted in the US versus in European communities. The second approach to combining phthalate metabolites used pretesting for statistical significance of concentrations of individual phthalate metabolites (one from each parent compound) and then created a score by summing the quartile of concentrations for each of the remaining metabolites of interest (starting

from zero for the lowest quartile) (Swan et al. 2005). The selection of variable subsets with pretesting (which sets some variables to have $\beta = 0$) and the use of combination metrics like the low or high molecular weight phthalates or the phthalate quartile score sum can alleviate some of the issues of highly correlated predictors. However, these approaches also require strong assumptions that may be inappropriate (Greenland 1994), and a loss of information that prevents these models from being extended to look at component-specific estimates or interactions.

*Challenges in implementing these modeling strategies*

With advances in statistical computing and freely available software such as R and WinBUGS, the limitation in fitting more sophisticated models when using biomarker data is shifting away from computation and custom programming and relies more on the judgment, experience, and technical comfort of the analyst. However, the additional assumptions of the Bayesian models require care that model results reflect the data rather than inappropriate prior values or model structures. The use of sensitivity analyses that vary prior values can help check the appropriateness of models and resulting interpretations.

We offer the following recommendation for the analysis of biomarker data, when there are multiple contaminants or other covariates that are chemically related or have common sources of exposure:

Step 1: Examine the correlation structure among exposures/predictors. Highly correlated predictors may require additional modeling strategies.

Step 2: Fit multiple-variable regression models using Bayesian methods:

(c) A simple Bayesian model that stabilizes highly correlated predictors by pulling effect estimates towards a prior. This is equivalent to ridge regression which is available in

many popular statistical packages. It may not be adequate if all effect size estimates for predictors are not expected to be similar.

(d) Bayes model with grouping: by using subject area knowledge, predictors can be grouped into subsets that are more reasonably expected to have exchangeable parameter estimates. This model can also incorporate additional prior information about the effect measures of predictors if it is available.

**Conclusions**

Our application is an example of the potential use of Bayesian regression for multiple correlated biomarker exposures. Models that include only one of a set of highly correlated predictors may be difficult to interpret, and OLS estimation in a full multiple linear model could be unstable. We suggest the additional use of simple Bayesian regression strategies to stabilize estimates and reduce variance in parameter estimation without relying on strong assumptions or the loss of information that can result from using summary measures. Although concentrations of several phthalate metabolites are significantly associated with reduced gestational age in single models, our data suggests that no single metabolite explains the observed association. Instead, we believe that larger (negative) magnitude estimates for MEHP, MEHHP (MEOHP), and MCPP indicate that the association with reduced gestational age may be due to exposure to DEHP, DnOP, and potentially other higher molecular weight phthalates.

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**Declaration:** The authors declare no competing interests.

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# Appendices

## "Bayesian models for correlated biomarkers of exposure: multiple phthalate metabolites and association with gestational age"

## Table of Contents

*Assessing potential non-linear associations*

Our previous results modeled concentrations of individual metabolites of DEHP one at a time and found both a linear effect and an increase across quartiles that was only statistically significant in the fourth quartile (Whyatt et al. 2009). To pursue this further, we plotted concentrations of each of the phthalate metabolites one at a time with penalized splines to allow for non-linear associations while controlling for premature rupture of membranes and planned Cesarean section. Penalized spline regression (Ruppert et al. 2003) results for concentrations of single DEHP metabolites and covariates show an association that is inverse and linear, lying alongside the line representing the strictly linear model, except for variation at the ends of the distribution where there are very few observations reflected in the widening of the 95% credible interval. Results for MEHHP, the single metabolite with the largest absolute effect size, are shown in Figure E1.

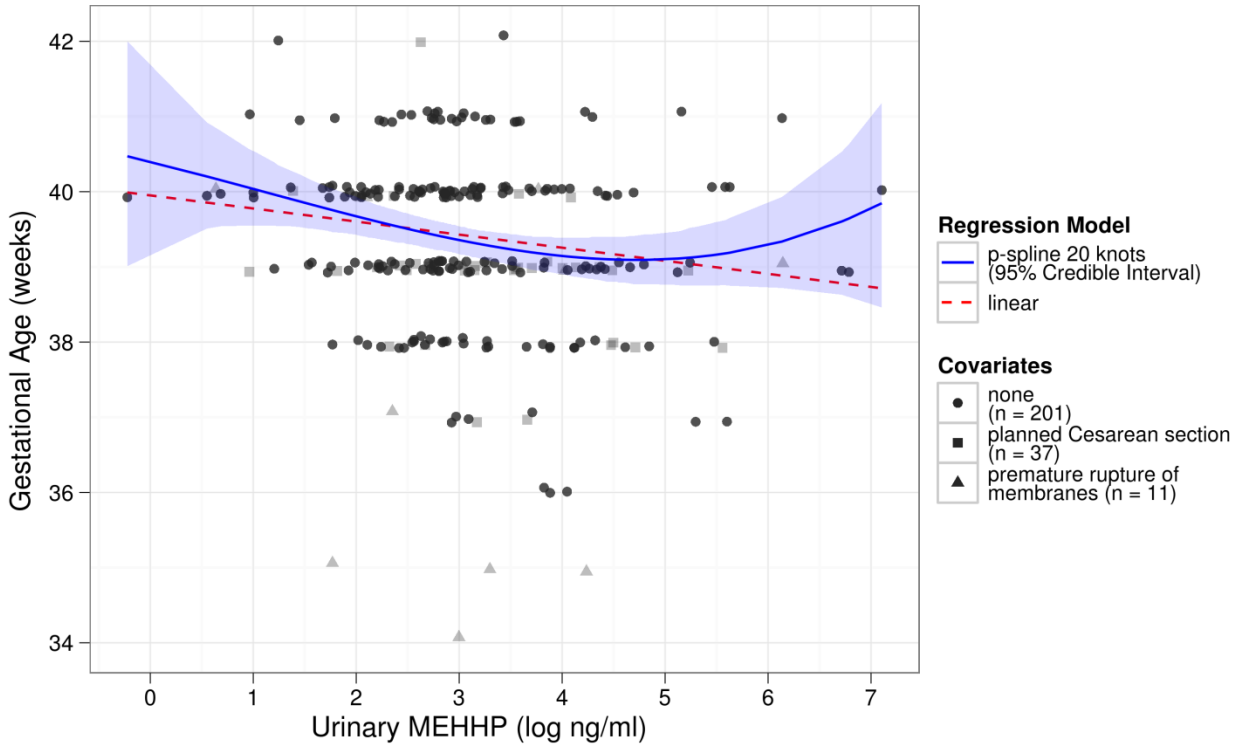


**Figure E1. Penalized spline (with 95% credible interval shaded) shows a decline in gestational age with increasing exposure to MEHHP that is consistent with a linear model (shown as a dashed line).**
The regression model predictions are shown for the data subset without controlled covariates.

*Selection of priors and sensitivity analysis*

The estimated regression coefficients for our set of predictors in a ridge regression model at varying levels of shrinkage parameter (λ) are shown in Figure E2. A model fit without shrinkage applied on regression coefficients (λ = 0) is equivalent to an ordinary least squares (OLS) linear regression model. Although the estimates based on the OLS are asymptotically unbiased, it has the widest range for the magnitude of parameter estimates from the phthalates and other covariates. As λ increases, the estimates of regression coefficients are penalized to shrink toward zero, and estimated prediction error is reduced to a point, measured here by a local minimum for the generalized cross-validation statistic. Notably, as the shrinkage increases the highly correlated predictors MECPP and MEHHP are pulled towards zero more rapidly than the uncorrelated other covariates. As λ increases further, the magnitudes of coefficient estimates continue to reduce, but the estimated prediction error increases as well. An excess of shrinkage will pull parameter estimates too far towards zero with infinite shrinkage equivalent to an intercept-only model which will have poorer model fit than a more judicious use of predictor variables. Because the generalized cross-validation (GCV) statistic is easily computed, it was selected as a metric to select the amount of shrinkage that corresponded to the optimal bias-variance tradeoff by minimizing the generalized cross-validation based prediction error.

Because the amount of shrinkage in the Bayesian models we used is a function of both the data and the priors, it is important to conduct sensitivity analyses to understand the role of prior distribution parameters in these results. Our selection of prior variance values from our own dataset corresponds to the previously described “semi-Bayes” strategy (Greenland 1994).

Several previously published studies that advocated using Bayesian models for multiple exposure models were based on applications with multiplicative models for dichotomous outcomes

such as cancer, infant mortality, and retinal degeneration (De Roos et al. 2001; De Roos et al. 2003; Greenland 1994; MacLehose et al. 2007; Momoli et al. 2010; Witte et al. 1994). The authors of these papers advocate selection of a prior variance based on a reasonable multiplicative range for parameter estimates (e.g. a belief that 95% of estimates will be within 16-fold of each other). Unfortunately, this appealing and understandable interpretation isn't applicable to the additive model used with our continuous outcome of gestational age in weeks. The optimization of the generalized cross-validation statistic offers an alternative approach for selecting a prior variance which has a basis in reducing model prediction error.

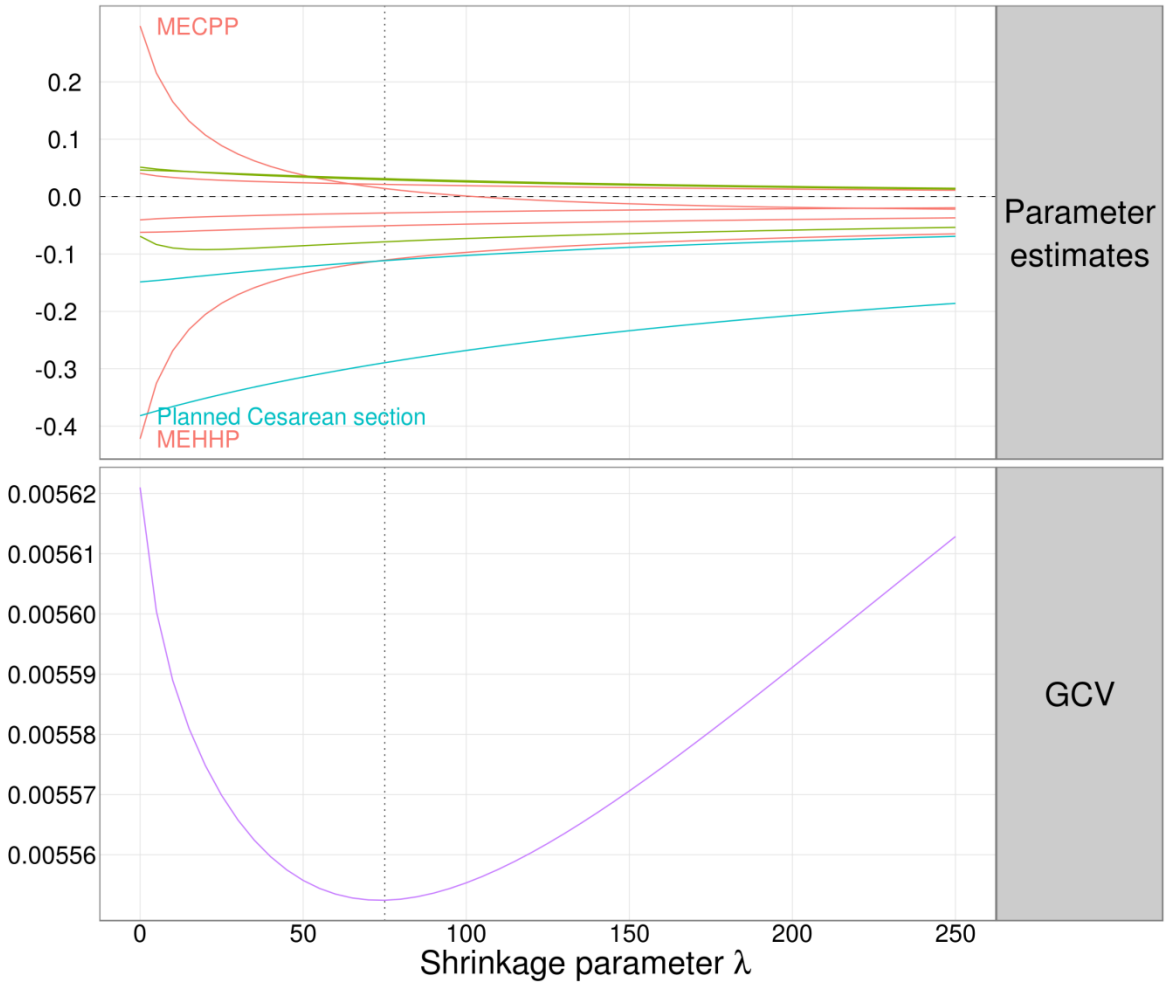


**Figure E2. Lines in the top panel show parameter estimates and the bottom panel shows the generalized cross-validation statistic (GCV) with different amounts of shrinkage, λ, from ridge regression.**

Line labels show that MECPP and MEHHP, the two highly correlated DEHP metabolites with the most widely spread estimates at $\lambda = 0$ are pulled towards 0 more rapidly with increasing $\lambda$ than other variables, particularly planned Cesarean section. The vertical dotted line corresponds to the level of $\lambda$ which minimizes the GCV.

*R Code to fit these simple Bayesian models*

The following annotated code runs two simple Bayesian multivariable regression models for a continuous outcome that has been centered; (1) a simple model we call “Bayes-Ridge” with priors centered on zero and a fixed error term and (2) “Bayes-Group”, a model with a more flexible error structure and prespecified grouping of similar predictors. This code was tested on a Windows 7 system using both R version 2.12.2 (R Development Core Team 2011), run as administrator with packages “arm” (Gelman et al. 2011), “MASS” (Venables and Ripley 2002), and their dependencies including “R2WinBUGS” (Sturtz et al. 2005) installed, as well as WinBUGS 1.4.3 (Lunn et al. 2000). The code assumes that a dataset has already been loaded in R and is named “dataframe”.

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Witte JS, Greenland S, Haile RW, Bird CL. 1994. Hierarchical regression analysis applied to a study of multiple dietary exposures and breast cancer. Epidemiology 5(6): 612-621.

*R code for WinBUGS models*

```
#####################################################
# R code for WinBUGS models
# with correlated predictors and a continuous outcome

# February 2011
# Tested on Windows XP with R 2.12.2 and WinBUGS 1.4.3
#####################################################

## INSTRUCTIONS
# This file fits two simple bayesian models as described in comments below

# We assume the user starts with a data frame in the
# local environment named 'dataframe' and has installed WinBUGS
# available at www.mrc-bsu.cam.ac.uk/bugs/

# we assume that the centered outcome is the first column
# and that all remaining columns are standardized predictors
# note that the code as written does not accommodate extraneous variables
# and it restricts to complete data

# For the Bayes model which assumes grouping of covariates,
# the model expects the columns to be sorted so that the covariates in
# groups are together. In the following code, we assume 3 groups
# with 5, 3, and 2 covariates respectively.

## load libraries
library(arm) #also loads R2WinBUGS
library(MASS)#contains lm.ridge function

## local functions
#
# ridge.search.gcv
# Description - function to explore local values around crude best GCV
# Arguments:
# ridgecall- a symbolic description of the formula to be fitted,
#            the linear predictor. see ?formula.
# data     - specify the dataset, which is of class data.frame
# lambda   - vector of initial shrinkage parameter values over which to
#            search. Best initial value is the one which minimizes GCV
#            (generalized cross validation). Must be non-negative.
# lambdasearch - distance +/- from best initial lambda to explore,
#            over steps of 0.1. default is 10.
# verbose  - option to print intermediate results:  best initial lambda,
#            best lambda on local search, and regression coefficients
#            from ridge model; default is FALSE.
# Results:
# returns the optimal estimated shrinkage parameter lambda based on GCV.

ridge.search.gcv <- function(
      ridgecall, data, lambda, lambdasearch = 10, verbose = F){
 lambda.gcv <- lm.ridge(ridgecall, data = data, lambda = lambda)
 best.lambda1 <- which.min(lambda.gcv$GCV)
 if(verbose){
  print(paste("best initial lambda is", names(best.lambda1)))
```

```
 }

 # create a series of lambda values nearby (non-negative)
 local.lambda <- round(lambda.gcv$lambda[best.lambda1], 0.1)
 local.lambda <- seq(max(0, local.lambda - lambdasearch),
  local.lambda + lambdasearch, 0.1)
 lambda.refit <- lm.ridge(ridgecall, data = data, lambda = local.lambda)

 # warn if lowest GCV is at the boundary of tested lambdas
 if(which.min(lambda.refit$GCV) %in% c(1, length(local.lambda))){
  warning("lowest GCV at boundary,\n try larger lambdasearch")
 }
 best.lambda2 <- which.min(lambda.refit$GCV)
 if(verbose){
            print(paste("best lambda on local search is", names(best.lambda2)))
  print(coef(lambda.refit)[best.lambda2, ])
 }
 invisible(as.numeric(names(best.lambda2)))
 # returns the optimal lambda without printing
}

# rsd: Extracts the residual standard deviation (from a linear model object)
# Arguments:  x which is an object of class "lm"
rsd <- function(x) summary(x)$sigma

#####################################
## defining the model formula
# (column 1 ~ remaining columns)
formula.bugs <- formula(dataframe)
# remove the intercept term
formula.bugs <- update(formula.bugs, ~ -1 + .)
# IMPORTANT: this is the formula that will be fit for the Bayes models
print(formula.bugs)

# check that there is a data.frame named “dataframe”
if(!exists("dataframe")) warning("need an object named 'dataframe'")
if(!is.data.frame(dataframe)) warning("'dataframe' must be a data.frame")
# restrict the dataset to complete cases
dataframe <- dataframe[complete.cases(dataframe), ]

#####################################################
# linear regression; no intercept model formula
BR <- lm(formula.bugs, data = dataframe)
display(BR)

# prespecified model errors (sigma.y.fixed)
# and model prior variance (tau.fixed)
sigma.y.fixed <- round(1/(rsd(BR)^2) , 2)
sigma.y.fixed # model error from linear model
lambda.gcv <- ridge.search.gcv(formula.bugs, dataframe,
 lambda = c(0, exp(1:10)), lambdasearch = 100, verbose = T)
tau.fixed <- round(1 / (rsd(BR)^2/lambda.gcv), 2)
tau.fixed # inverse of ratio of rsd^2 and lambda.gcv

# program a bugs file “BR.bug”, saved to working directory
# uses the gcv from ridge regression to select the amount of shrinkage
cat("# Model BR.bug
```

```
 model
 {
 for (i in 1:n){
  y[i] ~ dnorm(mu[i], ", sigma.y.fixed, ")
  mu[i] <- inprod(x[i, ], beta[])
 }
 ### Priors
 for (p in 1:10)(beta[p] ~ dnorm(0, ", tau.fixed, "))
 }",
 sep = "", file = "BR.bug")
file.show("BR.bug")

y <- dataframe[, 1] # outcome
n <- length(y)  # number of observations
x <- as.matrix(dataframe[, -1]) # design matrix
j <- dim(x)[2] # number of parameters in model matrix
BR.data <- list ("n", "y", "x")
# starting values for parameters; set to 0
BR.inits <- function()list(beta=rep(0, j))
BR.parameters <- c("beta")

# run in winbugs1.4 – this can take a few seconds
BR.out <- bugs(BR.data, BR.inits, BR.parameters,
 "BR.bug", n.chains=3, n.iter=500, debug = FALSE)

# display the results on console using variable names from dataframe
dimnames(BR.out$summary)[[1]][1:j] <- dimnames(x)[[2]]
print(BR.out, digits = 2)
#plot(BR.out)

#####################################################
# model BG (Bayes-Group)
# like model P2 in MacLehose et al. 2007, it places a distribution on both
# the model error (sigma.y with a minimally informative prior)
# and the variance of the prior on parameters (tau).
# it also separates covariates into 3 groups (similar to Momoli et al. 2010).
# these are mutually exclusive groups of our 1:p predictors
# here we use 1:5, 6:8, and 9:10
# otherwise the structure of the R and bugs code is
# similar to Bayes-Ridge above

# revisiting the form of the linear predictor
formula.bugs
# linear regression of our no-intercept model formula
BG <- lm(formula.bugs, data = dataframe)
display(BG)

# prespecified model errors (sigma.y.fixed)
# and model prior variance (tau.fixed)
sigma.y <- round(1/(rsd(BG)^2) , 2)
sigma.y # model error if you were using ridge
lambda.gcv <- ridge.search.gcv(formula.bugs,
 dataframe, lambda = c(0, exp(1:10)), lambdasearch = 100, verbose = T)
tau.fixed <- round(1 / (rsd(BG)^2/lambda.gcv), 2)
tau.fixed # inverse of ratio of rsd^2 and lambda.gcv

# program a bugs file “BG.bug”, saved to working directory
```

```
# uses the gcv from ridge regression to select the amount of shrinkage
cat("#
 # Model BG.bug
 # like model P2 in MacLehose, it has a prior on both
 # model error, tau.y,
 # and on the prior variance for betas, tau
 # it also has three separate group means for regression parameters;
 model
 {
 for (i in 1:n){
  y[i] ~ dnorm(mu[i], sigma.y)
  mu[i] <- inprod(x[i, ], beta[])}
 ### Priors by group
 for (p in 1:5)(beta[p] ~ dnorm(theta0, tau))
 for (p in 6:8)(beta[p] ~ dnorm(theta1, tau))
 for (p in 9:10)(beta[p] ~ dnorm(theta2, tau))

      theta0 ~ dnorm(0, 1)
 theta1 ~ dnorm(0, 1)
 theta2 ~ dnorm(0, 1)
 tau ~ dgamma (", tau.fixed, ", 1)
 sigma.y <- pow(inv.sigma.y, -2)  # inv.sigma.y = 1/sigma^2
 inv.sigma.y ~ dunif(0, 1000)  # noninformative prior
 }",
 sep = "", file = "BG.bug")
file.show("BG.bug")

## the entire code, as written, fits 2 Bayesian models
## if not run earlier, run the following 4 commented lines
# y <- dataframe[, 1]
# n <- length(y)
# x <- as.matrix(dataframe[, -1])
# j <- dim(x)[2] #  number of parameters in model matrix

BG.data <- list ("n", "y", "x")
BG.inits <- function() list(beta=rep(0, j), sigma.y = 1,
      tau = tau.fixed, theta0 = 1, theta1 = 1, theta2 = 1)
BG.parameters <- c("beta", "sigma.y", "tau", "theta0", "theta1", "theta2")

# run in winbugs1.4
# this can take a few moments while the model updates
BG.out <- bugs(BG.data, BG.inits, BG.parameters,
 "BG.bug", n.chains=3, n.iter=750, debug = F)

# display the results on console using variable names from dataframe
dimnames(BG.out$summary)[[1]][1:j] <- dimnames(x)[[2]]
print (BG.out, digits = 2)
#plot (BG.out)
# End of Code ###################################
```

**Chapter 6: Prenatal exposure to butylbenzyl phthalate and early eczema in an urban cohort**

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**Abstract**

Background: Recent cross-sectional studies suggest a link between butylbenzyl phthalate (BBzP) in house dust and childhood eczema.

Objectives: To evaluate whether monobenzyl phthalate (MBzP), the main BBzP metabolite in maternal urine, during pregnancy is associated prospectively with eczema in young children, and whether this association varies by the development of seroatopy in the child.

Methods: MBzP was measured in spot urine samples collected during the 3rd trimester of pregnancy from *n*=407 African American and Dominican women residing in New York City in 1999-2006. Repeated questionnaires asked mothers whether their doctor ever said their child had eczema. Child blood samples at ages 24, 36, and 60 months were analyzed for total, anti-cockroach, dust mite, and mouse IgE. Analyses included a multinomial logistic regression model for early and late onset eczema versus no eczema through age 60 months. Relative risks were estimated with multivariable modified Poisson regression.

Results: MBzP was detected in >99% of samples (geometric mean 18.9, interquartile range 8.0, 43.2 ng/ml). By 24 months, 30% of children had developed eczema, with the proportion higher among African Americans (48%) than Dominicans (21%) ($p<0.001$). MBzP concentrations were associated positively with early onset eczema (relative risk 1.52 for eczema by 24 months, 95%CI [1.21, 1.91], $p<0.001$, *n*=376) for an interquartile range increase in log MBzP concentration, adjusting for specific gravity, sex, and ethnicity. MBzP was not associated with seroatopy, nor did seroatopy modify the MBzP and eczema association.

Conclusions: Prenatal exposure to BBzP may influence the risk of developing eczema in early childhood.

**Introduction**

Eczema, a type of persistent itchy skin rash, in children is considered an early manifestation of allergic disease, and can mark the beginning of the "atopic march" (Leung et al. 2004). Although exposure to allergens may be important in the development of eczema, exposure to other environmental agents has been implicated as well. For example, in cross-sectional studies, environmental tobacco smoke, semi-volatile contaminants of indoor air such as propylene glycol, glycol ether and butylbenzyl phthalate (BBzP), and residential renovation around the time of birth have been associated with eczema in young children (Bornehag et al. 2004; Choi et al. 2010; Herbarth et al. 2006; Kramer et al. 2004). Results from a 2003 U.S. national survey suggest that living in urban areas, African American race, higher parental education, and child-care attendance are associated with an increased prevalence of childhood eczema (Shaw et al. 2011). Further, either atopic or nonatopic eczema (also referred to as intrinsic or nonallergic eczema) may develop in childhood. The two phenotypes differ in their risk factors and in their immunopathogenesis (Kusel et al. 2005).

Some phthalates are added to plastics to increase flexibility and are common components of consumer products including flooring materials and certain plastic household products. BBzP is a common component of vinyl flooring and is found frequently in house dust and indoor air (Adibi et al. 2008; Kolarik et al. 2008a). Monobenzyl phthalate (MBzP), the main BBzP metabolite, was detected in greater than 97% of urine samples in the U.S. representative 1999-2000 National Health and Nutrition Examination Survey (NHANES) (Silva et al. 2004), and detection in amniotic fluid indicates transplacental exposure during pregnancy (Wittassek et al. 2009). Although diet is believed generally to be the largest contributor to BBzP exposure in adults (Wormuth et al. 2006), in the Columbia Center for Children's Environmental Health (CCCEH) study we reported that

concentrations of BBzP collected in 48-hour personal air samples during pregnancy correlated with concentrations of MBzP in paired maternal spot urine samples (Spearman's Rho: 0.48, $p<0.05$, $n=62$) (Adibi et al. 2008). Hence, non-dietary routes, including inhalation, also may be important.

House dust concentrations of several phthalates were measured in a Swedish case-control study comparing case children with physician-confirmed persistent eczema, rhinitis, or wheezing without a cold, with asymptomatic controls. Median concentrations of BBzP in bedroom dust were significantly higher among children with physician diagnosed eczema and rhinitis versus healthy children with a positive dose-response trend in the odds ratio across quartiles of exposure (Bornehag et al. 2004). Polyvinyl chloride (PVC) flooring, considered an important source of phthalate plasticizers in the home, also was associated significantly with eczema and rhinitis. Additionally, higher concentrations of di(2-ethylhexyl) phthalate (DEHP), a common PVC plasticizer, were found in bedroom dust of cases with asthma compared with controls. The Swedish study design was replicated in Bulgaria where BBzP concentrations were higher in the house dust of children reporting wheeze or eczema, but the difference was not statistically significant (Kolarik et al. 2008b). While these findings are suggestive of a role of BBzP in the development of eczema, the use of urinary biomarkers offers a more direct measure of individual exposure than house dust by integrating ingestion, inhalation, and dermal absorption. In addition, longitudinal studies may offer further insight into the timing of exposure relative to disease onset.

The purpose of our study was to investigate whether prenatal measures of BBzP are associated with eczema and sensitization to common indoor aeroallergens in early childhood in an urban birth cohort. We hypothesized that prenatal BBzP would be associated with increased risk of eczema and elevated indoor allergen specific immunoglobulin (Ig) E.

**Methods**

As part of the CCCEH birth cohort, non-smoking pregnant Dominican and African American women were recruited during the third trimester and *n*=727 fully enrolled mother-child pairs were followed prospectively as previously described (Perera et al. 2003). Participants for the current analysis (*n*=407) had prenatal urinary phthalate metabolites measured (*n*=426/727) and responded to at least one questionnaire on eczema between child age 3 and 60 months. Study procedures, including questionnaires and collection of biologic samples, were explained to participants at enrollment and a signed consent, approved by the institutional review boards of Columbia University and the Centers for Disease Control and Prevention (CDC), was obtained.

Spot urine samples were collected during the third trimester (mean gestational age 34 weeks, interquartile range (IQR) 32 to 36 weeks) from 1999-2006 and analyzed for metabolites of BBzP and several other phthalates at the National Center for Environmental Health of the CDC as described (Adibi et al. 2008; Kato et al. 2005). The specific gravity of the urine was measured using a handheld refractometer (Atago PAL 10-S, Bellevue, WA).

Eczema was assessed by questionnaires administered to the mother by telephone and in-person at repeated visits by asking: “Has your doctor ever said that your child has eczema?” Four questionnaires were administered in each of the first two postnatal years and another four questionnaires between ages 24 and 60 months (for a maximum of 12 questionnaires). Two-thirds of participants answered at least 9 questionnaires over the 12 possible time-points. At age 60 months the International Study of Asthma and Allergies in Childhood (ISAAC) core eczema module (Asher et al. 1995) also asked “Has your child ever had an itchy rash which was coming and going for at least 6 months?”, and among those who responded positively “Has your child had this itchy rash at any time in the last 12 months?”

Sera were collected from the children during visits at ages 24, 36, and 60 months. Seroatopy was defined as a positive ( ≥0.35 IU/ml) specific IgE to cockroach, dust mite, or mouse in any available sample by ImmunoCap (Phadia, Uppsala, Sweden) (Chang et al. 2010; Donohue et al. 2008).

*Statistical analysis*

Descriptive statistics were compared among data subsets with t-tests and chi-square tests for continuous and categorical variables respectively. Urinary concentrations of MBzP were log transformed prior to analysis. Specific gravity was retained as a covariate in all models to account for variation in urinary dilution (Barr et al. 2005). One participant with a concentration of MBzP below the limit of detection (0.3 ng/ml; specific gravity 1.005) was excluded from further analysis because imputing a concentration of half the limit of detection and subsequent log transformation of the predictor created an influential point in the regression. Sensitivity analysis showed that parameter estimates excluding this sample did not differ from those in which 1 ng/ml was added to all concentrations prior to log transformation.

Eczema was reclassified as early onset eczema if it was reported on any questionnaire through 24 months of age. This combined variable for early onset eczema was restricted to children with at least one of four completed questionnaires in the first year, and one of four in the second year ($n$=376). Data on seroatopy using specific IgE were available for 94% of these children ($n$=355). Eczema was reclassified as late onset eczema if the first report of ever eczema was reported between age 24 months and 60 months among participants who completed at least one of four questionnaires in the first year, one of four in the second year, and one of four in the third through fifth year. A multinomial logistic regression model for early and late onset eczema

versus no eczema through age 60 months as the referent group was performed, restricted to participants who remained enrolled through 60 months (*n*=339).

A modified Poisson regression was used to generate relative risk (RR) regression parameters and variance estimates (Zou 2004). Although this is a multiplicative model, potential interactions between risk factors were tested as a departure from additivity using the relative excess risk due to interaction (RERI) (Rothman et al. 2008), with 95% confidence intervals from percentiles of 10000 bootstrap samples (Knol et al. 2007). Statistical analyses were conducted in R version 2.13.0 (R Development Core Team 2011) with the "effects" package for the confidence interval on a multinomial model and "ggplot2" package for graphics (Fox and Hong 2009; Wickham 2009).

## Results

### *Cohort characteristics*

Demographic characteristics of the *n*=407 mother-child pairs included in analyses and the remainder of the CCCEH cohort are described in Table 1. Participants included in analyses did not differ in major demographic characteristics from the remainder of the CCCEH cohort, except that they were less likely to have prenatal exposure to environmental tobacco smoke (31% versus 39%) and had less seroatopy at 24 months (8% versus 17%) in available samples; although, seroatopy did not differ at 36 or 60 months or when combined as described in methods. MBzP was detected in all except one urine sample and concentrations ranged widely (geometric mean (GM) of 18.9, IQR [8.0, 43.2] ng/ml). MBzP concentrations were higher among African American (GM 25.5, IQR [12.1, 49.2] ng/ml) than Dominican women (GM 16.2, IQR [6.8, 36.6] ng/ml) ($p<0.001$). There was no association between prenatal MBzP urinary concentrations and self report of maternal asthma (GM 20.8 and 18.3 ng/ml for asthmatic and non-asthmatic women, $p=0.36$).

*Eczema characteristics*

By child age 24 months, 30% of mothers had reported at least once that their doctor had ever said that their child had eczema, with a higher proportion among African American (48%, *n*=62/129) than Dominican (21%, *n*=51/247) children. Among Dominicans, the proportion of mothers reporting eczema in their children by language of questionnaire administration (English versus Spanish) was the same. There was no difference in the proportion of children with ever-eczema by child sex overall (32% female, 28% male, p=0.37) or after stratifying by race/ethnicity (not shown). The proportion of children with ever eczema generally increased with the child's age, from 10% at age 3 months to 28% at age 60 months. However, women were sometimes inconsistent in their report of their child's eczema. Among those who had a positive report of ever-eczema (at any of the 12 questionnaire time-points), 59% of subsequent questionnaires were also positive.

*MBzP and eczema*

Adjusted RR estimates of child ever-eczema for an IQR increase in prenatal MBzP urinary concentrations at each of 12 questionnaire time-points are shown in Figure 1. Relative risks appear to be consistently positive with larger magnitudes, suggesting a stronger association, at the earlier ages. Prenatal MBzP urinary concentration was associated positively with any report of eczema by 24 months (RR 1.52, 95%CI [1.21, 1.91], p<0.001, *n*=376) for an IQR increase in log MBzP concentration after adjusting for specific gravity of the urine, sex, and ethnicity. The parameter estimate for child's sex was not significant in the multivariable model with a RR for males versus females (reference) of 0.82 (95%CI [0.60, 1.10], p=0.18). The prenatal MBzP estimate for any

report of eczema by 24 months had a similar magnitude in a logistic regression model (OR 1.99, 95%CI [1.34, 2.97], *n*=376) as in a multinomial analysis separately fitting ORs for inconsistent reporters who said no ever-eczema after having said yes ever-ezcema (OR 1.98, 95%CI [1.23, 3.17], *n*=63) and for consistent reporters (OR 2.02, 95%CI [1.19, 3.43], *n*=50) versus none (*n*=263). To determine whether the association between prenatal MBzP concentrations and eczema was greater among children with early onset (by 24 months) versus late onset eczema, multinomial analyses were performed (*n*=339) after reclassifying eczema as early (29%, *n*=100), late (13%, *n*=43), or none (58%, *n*=196). MBzP concentration was associated positively with early onset eczema (adjusted OR 1.91, 95%CI [1.23, 2.97] for an IQR change; Figure 2). There was no association between MBzP and late onset eczema, with an adjusted OR of 0.90 (95%CI [0.51, 1.58]) for an IQR change in log MBzP from the multinomial analysis. Moreover, there was no association between MBzP concentration and the recent symptom report at 60 months of “itchy rash at any time in the past 12 months that was coming and going for at least 6 months” (RR 1.23, 95%CI [0.82, 1.86], p=0.31, *n*=341).

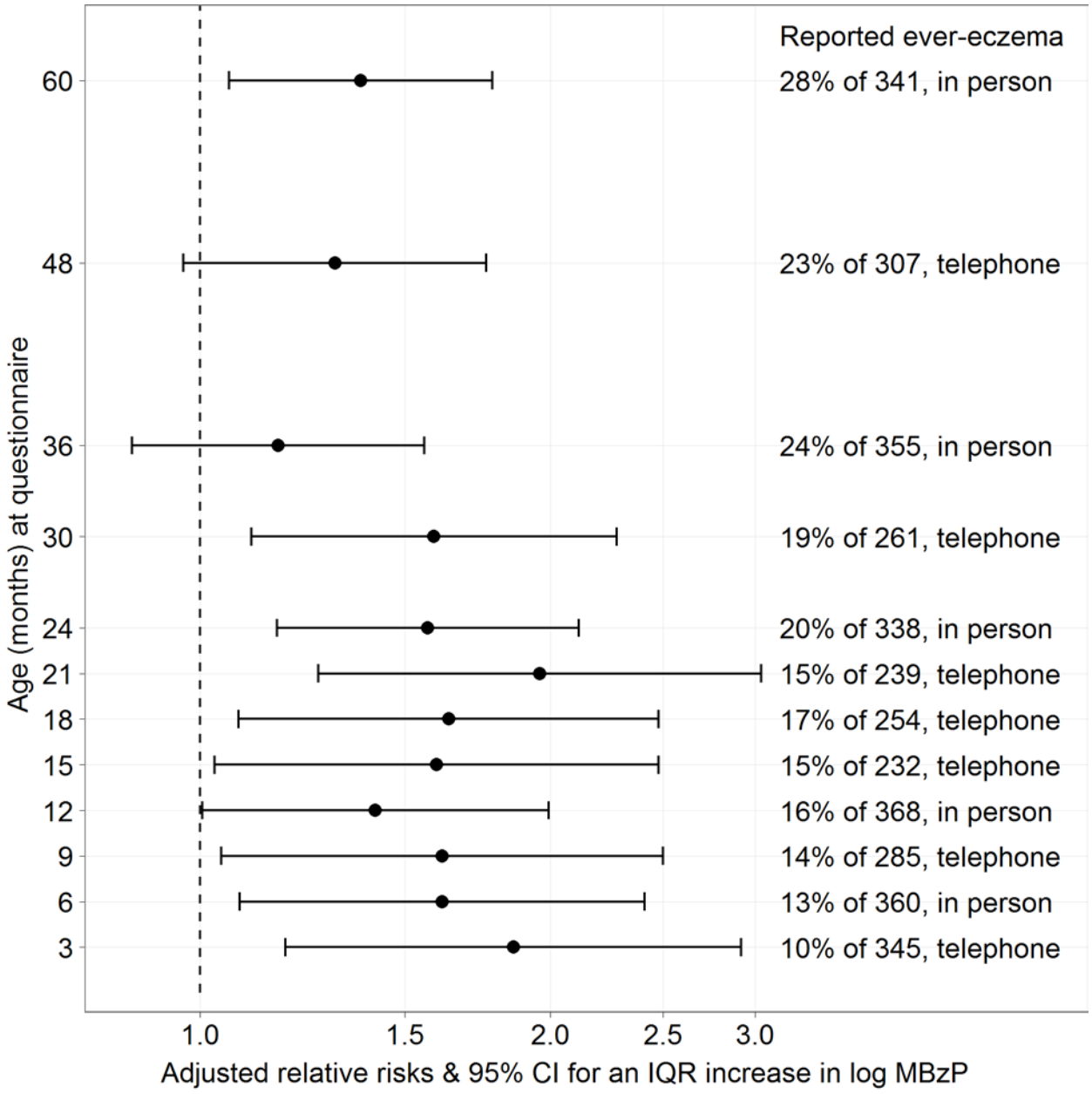


**Figure 1. Relative risk estimates of child ever-eczema for an interquartile range (IQR) increase in prenatal log MBzP urinary concentration adjusting for specific gravity, ethnicity, and sex from separate regression models using questionnaire data collected at 12 different ages in person and by telephone (*n*=407).**

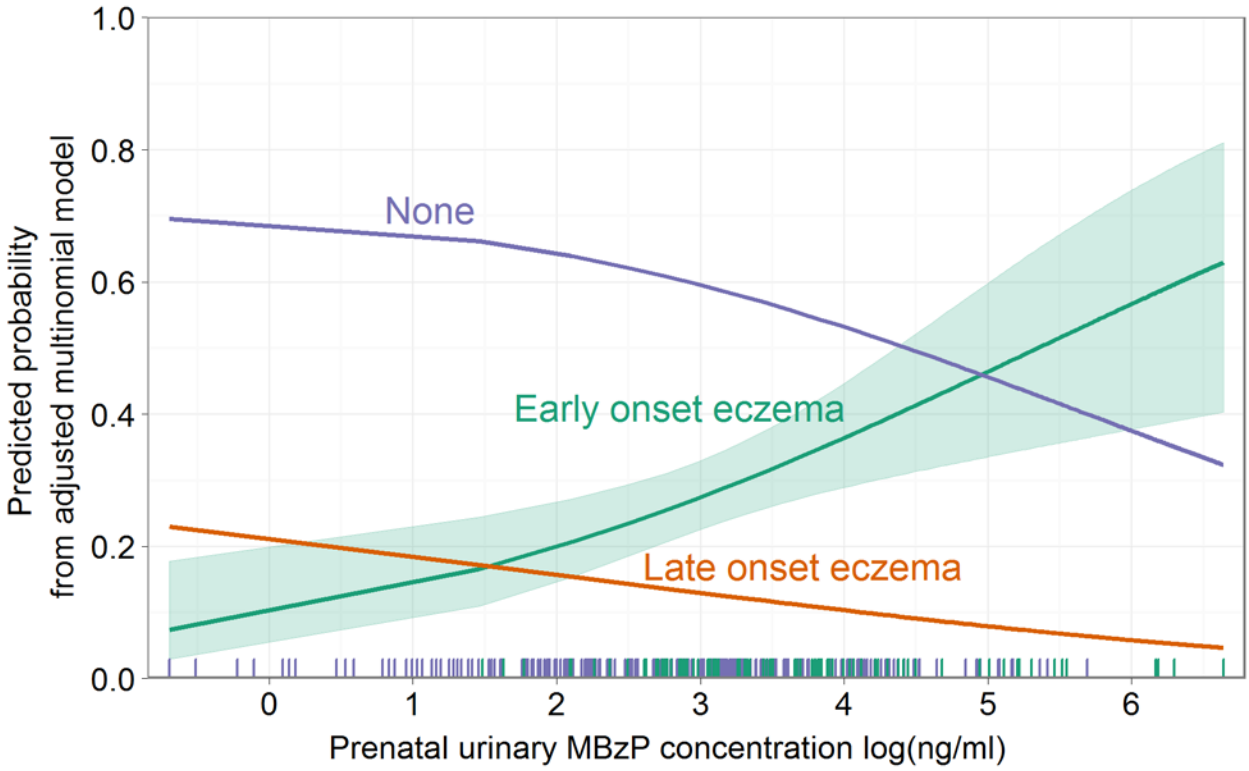


**Figure 2. Probability of no eczema, early onset eczema (by 24 months), and late onset eczema (30 to 60 months).**
Each line is the modeled probability of belonging to a group for a given log concentration of MBzP and the mean specific gravity, sex, and maternal ethnicity. A 95%CI is shown for the early onset eczema group and the rug along the x-axis is colored by the predicted outcome for each observation ($n$=339).

*MBzP and eczema by ethnicity*

Because previously we reported that eczema may vary by race/ethnicity (Donohue et al. 2008), we sought to understand better whether the association between phthalates and eczema was modified by ethnicity. Maternal African American versus Dominican ethnicity was a significant predictor of report of eczema by 24 months (RR 2.09, 95%CI [1.53, 2.86], p<0.001) after adjusting for MBzP concentration, specific gravity, and sex. There was a consistently higher probability of eczema for African Americans across the range of urinary concentrations of MBzP. Both ethnic groups had similar slopes for the exposure-response curve in stratified analysis, indicating a lack of multiplicative interaction (Figure 3). However, there was a nonsignificant greater than additive

interaction between African American ethnicity and each IQR increase in MBzP urinary concentration (RERI 0.54, Bootstrap 95%CI [-0.07, 1.39]).

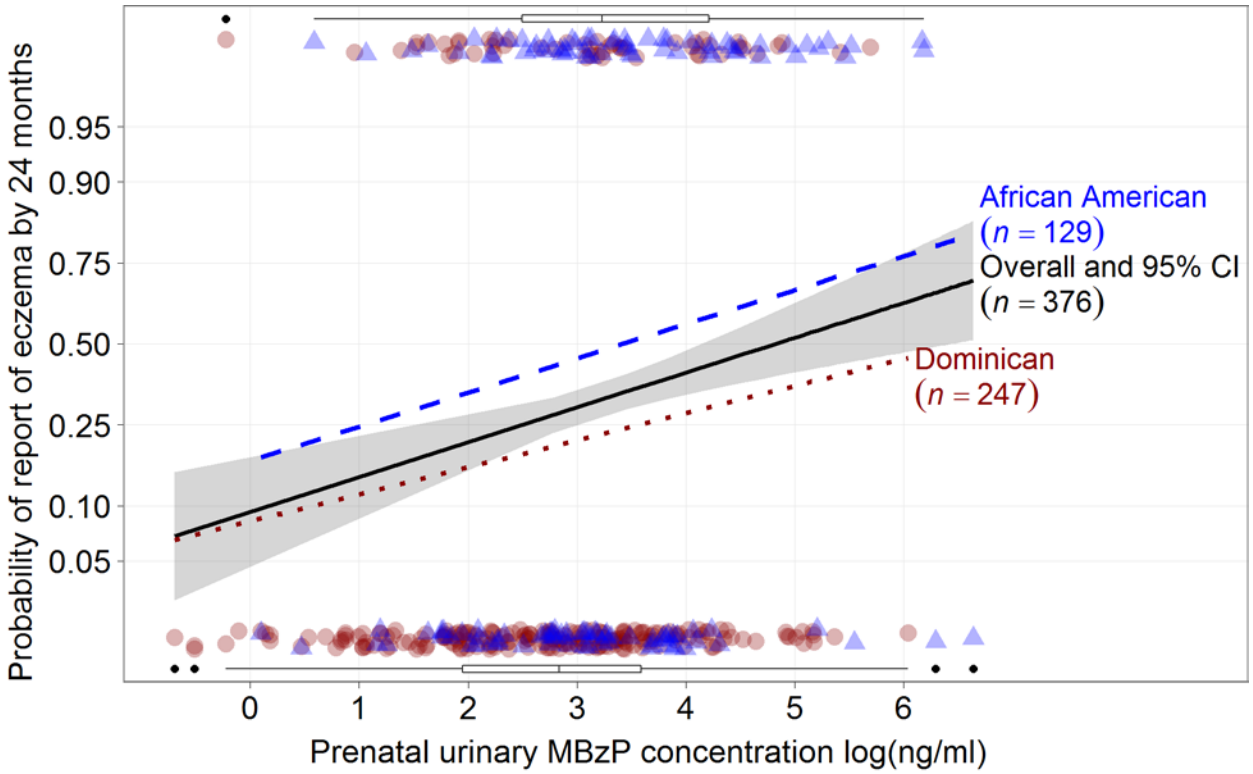


**Figure 3. Association between prenatal urinary concentration of MBzP and report of eczema by 24 months plotted as probability from a multivariable logistic regression adjusted with mean specific gravity and sex.**
The y-axis is shown on the logit scale. The overall cohort model is shown in black with 95%CI in grey. The ethnic subsets are shown in dashed and dotted lines for African Americans and Dominicans. Although the baseline probabilities differ in the two subsets, the overall association remains positive with increasing urinary concentrations of MBzP.

*MBzP, eczema, and seroatopy*

A total of 26% (*n*=91/355) of children were classified as seroatopic to indoor aeroallergens at age 24, 36, or 60 months. Among children with eczema by 24 months, 29% (*n*=31/106) were seroatopic and 71% (*n*=75/106) were non-seroatopic. This proportion of seroatopics was similar among children without eczema (24%, *n*=60/249) (p=0.31). Children with eczema by 24 months

on average developed higher total IgE levels at age 60 months compared to children without eczema, regardless of whether they were defined as non-atopic (GM: 39 IU/ml, *n*=67 versus 24 IU/ml, *n*=152; p=0.016) or seroatopic to indoor aeroallergens based on their age 60 month specific IgE values (GM: 207 IU/ml, *n*=22 versus 107 IU/ml, *n*=50; p=0.056).

There was no observed association between prenatal MBzP urinary concentration and seroatopy to indoor aeroallergens (RR 0.89, 95%CI [0.68, 1.16], p=0.81, *n*=355). Similarly, there was no observed association between MBzP concentration and log total IgE at 60 months ($\hat{\beta}$ -0.14, 95%CI [-0.41, 0.13], p=0.29, *n*=306) for an IQR change in MBzP concentration controlling for specific gravity, ethnicity, and sex. We also examined whether the association between prenatal MBzP urinary concentration and eczema differed by seroatopy to any one of three indoor aeroallergens. There was no significant interaction between seroatopy and higher MBzP concentration (continuous term) in predicting eczema using the RERI to test for departures from additivity, indicating that the MBzP urinary concentration and eczema association did not significantly vary by seroatopy (RERI 0.13, Bootstrap 95%CI [-0.29, 0.41]).

*Other phthalates and eczema*

Although urinary concentration of the metabolites of several other phthalates are correlated moderately with MBzP, in models with both prenatal MBzP and mono-*n*-butyl phthalate (a major metabolite of di-*n*-butyl phthalate and a minor metabolite of BBzP) or mono(2-ethyl-5-hydroxyhexyl) phthalate (metabolite of DEHP) urinary concentrations, only MBzP was a significant predictor of report of eczema by 24 months.

**Discussion**

We report here a novel association between prenatal urinary concentrations of MBzP, the main metabolite of BBzP, and the subsequent report of childhood eczema among African American and Dominican children in New York City. The association appears to be primarily among those with early onset eczema, reported by 24 months of age. Although the proportion of children with eczema is higher in African Americans than Dominicans, in both groups there is a similar positive association between prenatal urinary concentrations of MBzP and early onset eczema. No association was observed between prenatal urinary concentration of MBzP and seroatopy, as measured by specific IgE against three indoor allergens. These prospective results extend previous cross-sectional results nested in a Swedish cohort linking house dust concentrations of BBzP and eczema in a dose-response manner (Bornehag et al. 2004). While Swedish case children with physician-diagnosed eczema or rhinitis had statistically significantly higher BBzP concentration in their bedroom dust than controls, consistent with the findings here, there was no difference in BBzP concentration between atopic and non-atopic cases as classified by specific IgE (Bornehag et al. 2004).

One particular strength of the current study is the use of a urinary biomarker of phthalate exposure that has been shown previously in this population to have a good reproducibility over late pregnancy (ICC=0.66 for MBzP sampled 2-4 times over 6 weeks) (Adibi et al. 2008). In addition, the prospective birth cohort design allows a clear temporal order in which the measure of prenatal exposure precedes the development and subsequent report of children's health outcomes. It also has enabled us to evaluate the association between prenatal exposure to BBzP and early versus late onset eczema based on the age at first report. We observed that associations between prenatal urinary concentrations of MBzP and eczema had larger effect size estimates on questionnaires

collected at earlier ages than those after 24 months. In our multinomial analysis, MBzP urinary concentration was associated with early but not late onset eczema (first report after 24 months) relative to those in the no eczema group. Neither of the two major risk factors for early onset eczema, MBzP concentration and African American race/ethnicity, were associated with late onset eczema. Furthermore, fitted values from the full multinomial model did not predict any members of the late eczema group. Because late eczema was less common (13%, $n$=43/339), this finding may have been due partially to the instability of estimating associations for this small group and may not indicate that late onset eczema is a different clinical phenotype in this cohort.

Given the known association between eczema and atopy, we also hypothesized that exposure to BBzP would be associated with seroatopy to three indoor aeroallergens. This hypothesis was based on experimental evidence with other phthalates that may have relevance to BBzP. These include ex vivo experiments on human peripheral blood mononuclear cells incubated with DEHP, or its hydrolytic metabolite mono(2-ethylhexyl) phthalate (MEHP), that produced greater proallergic cytokine levels and histamine release (Glue et al. 2002; Glue et al. 2005; reviewed in Kwak et al. 2009). In addition, prenatal and neonatal exposure of mice to DEHP induced an atopic dermatitis-like phenotype in dust mite sensitized offspring that was characterized by greater local expression of the proallergic chemokine eotaxin (Yanagisawa et al. 2008). Interestingly, another phthalate, di-*n*-butyl phthalate, has been shown to upregulate proallergic skin hypersensitivity reactions via thymic stromal lymphopoietin (TSLP), a cytokine known to activate the maturation of dendritic cells and recently associated with eczema, in mice (Larson et al. 2010; Shigeno et al. 2009).

However, we observed that in this cohort MBzP urinary concentration was not associated with seroatopy, despite its association with eczema, suggesting that exposure to BBzP may

increase the risk of eczema through a non-allergic mechanism. This finding may be consistent with two studies in which BBzP failed to act as an adjuvant in mouse models of allergic sensitization (Dearman et al. 2009; Larsen et al. 2003). While eczema is considered frequently an early indicator of IgE-mediated allergic diseases (Leung et al. 2004), it should be noted that eczema among non-atopic children also is quite prevalent. As high as two-thirds of children with eczema are nonatopic in population-based studies (Flohr et al. 2004); in this cohort, 71% of those with reported eczema did not have seroatopy. Further, evidence suggests that immunopathogenesis of eczema can be triggered by mechanisms unrelated to allergic sensitization. For example, interleukin 31 has been implicated specifically in nonatopic eczema, and can induce the expression of several chemokines, including TARC/CCL17 (thymus- and activation-regulated chemokine) and MDC (macrophage-derived chemokine)/CCL22, important to the recruitment of inflammatory cells to the skin (Dillon et al. 2004; Schulz et al. 2007). Exposure to diisononyl phthalate, while not evaluated in this study, was shown likewise to induce the production of TARC/CCL17 and MDC/CCL22 in mice (Koike et al. 2010), although mediation via interleukin 31 has not yet been reported. Several phthalate metabolites, including MEHP and mono-*n*-octyl phthalate, but not MBzP, also have been shown to stimulate interleukin 8 in cell systems that could trigger a cascade of nonallergic proinflammatory events relevant to eczema (Jepsen et al. 2004). Finally, BBzP acts as an agonist of the peroxisome proliferator-activated receptors (PPARα and PPARγ) (Hurst and Waxman 2003). While the few studies that have examined immunologic responses to other PPAR agonists suggest that they suppress rather than induce skin inflammation (Jung et al. 2011; Staumont-Salle et al. 2008), it remains an open question whether interference with the PPARs could be one potential mechanism through which BBzP might affect the development of eczema.

The development of eczema related to phthalate exposure may vary by race/ethnicity in this cohort. The joint effect of an IQR increase in MBzP urinary concentration and African American race/ethnicity resulted in a relative risk estimate that was 0.54 higher (Bootstrap 95%CI on RERI [-0.07, 1.39]) than the expected sum of the main effects estimated in the absence of interaction, although this additive interaction did not reach statistical significance. We speculate that differences in dietary patterns as well as differences in the prevalence of indoor materials such as vinyl flooring made with BBzP, may contribute to the observed higher urinary concentrations of MBzP among African Americans. Differences in eczema prevalence by race have been reported previously. For example, in the 2003 National Survey of Children's Health, 9.7% of White-only and 15.9% of Black-only households reported eczema in the previous year. Race remained a significant predictor after controlling for a number of potential confounders also associated with higher eczema such as young child age, higher level of education, higher family income, and residence in a metropolitan area (Shaw et al. 2011).

We acknowledge several limitations. There was no assessment of early postnatal exposure to phthalates. Child eczema was assessed via questionnaire and therefore subject to a lack of standardization, misclassification or recall bias. The lack of full consistency in subsequent questionnaires after first report of ever-eczema may be an indication of this potential misclassification. However, our query: “Has your doctor ever said that your child has eczema?” has been validated by physician diagnosis in a study of Oregon school children (Laughter et al. 2000). We also have no reason to believe that any misclassification would be differential by phthalate exposure and thus should only bias the effect estimates towards the null. Although one could categorize participants by the first time when they reported ever-eczema, the exact age of onset only can be inferred indirectly from the questionnaire. Additionally, some of the differences

in the report of eczema by ethnicity may partially reflect differences in the understanding of what constitutes “eczema”, although there were no differences in report between English and Spanish speaking Dominican participants. In addition, the contribution of food allergy to MBzP-associated early eczema was not assessed and the higher total IgE among those with eczema than those without eczema suggests that sensitization to unmeasured allergens might be associated with eczema here. We also cannot rule out that children classified as non-atopic may later develop seroatopy.

The exposure monitoring found wide variation in prenatal exposure to BBzP in this urban population. Although dietary contamination as well as vinyl flooring are thought to be important sources for BBzP exposure, a dietary intervention failed to reduce exposure to BBzP (Rudel et al. 2011), and we are aware of no prior studies evaluating household interventions on phthalate containing products and materials. However, experience with other phthalates demonstrates that regulatory policies and market pressures can modify population-level exposures. For example, German industrial production of DEHP and exposure among students ages 20-29 were highly correlated (Helm 2007), and research suggests that a changing exposure profile for BBzP and other phthalates in that population may be attributed to a shifting regulatory environment (Wittassek et al. 2007).

**Conclusions**

Using a longitudinal urban US birth cohort, we found novel results that suggest that MBzP urinary concentrations measured prenatally are associated with early onset eczema. This finding was unrelated to seroatopy. Future studies may extend our findings using intervention designs to

test whether early-life exposure to BBzP can be reduced and whether this leads to a reduction in eczema, a very common childhood disease.

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**Chapter 7: Children's urinary phthalate metabolites and fractional exhaled nitric oxide in an urban cohort**

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Running title: Children's urinary phthalates and FeNO

**Abstract**

Rationale: Phthalates are widely used in consumer products, and exposure to several have been associated with respiratory symptoms and decreased lung function. Associations between children's phthalate exposures and fractional exhaled nitric oxide (FeNO), a biomarker of airway inflammation, have not been examined.

Objectives: We hypothesized that urinary concentrations of four phthalate metabolites would be associated with higher FeNO, and these associations would be stronger among children with seroatopy or wheeze.

Methods: In an ongoing birth cohort in New York City, 280 children without a cold had phthalate metabolites analyzed in spot urine samples collected within one week of an FeNO measure. Repeated sampling gathered a total of *n*=373 observations between ages 4.9-9.5 years and regression models used generalized estimating equations. Seroatopy was assessed by specific IgE to cockroach, dust mite, or mouse allergens. Wheeze in the past 12 months was assessed with a validated questionnaire.

Measurements and Main Results: Log-unit increases in urinary metabolites of diethyl phthalate (DEP) and butylbenzyl phthalate (BBzP) were associated with a 6.1% (95% confidence interval [CI] 0.2%, 12.4%) and 8.2% (95% CI 1.7%, 15.1%) increase in FeNO, respectively, adjusting for the other phthalate metabolites, specific gravity, seroatopy, sex, age, race/ethnicity, and ambient nitric oxide. There was no association between urinary metabolites of di(2-ethylhexyl) phthalate (DEHP) or di *n*-butyl phthalate (DnBP) and FeNO in the same model. There was no significant interaction by seroatopy. The BBzP metabolite association was significantly stronger among children who wheeze (p=0.04).

Discussion: Results suggest independent associations between exposures to DEP and BBzP and FeNO in a cohort of inner-city children. These two ubiquitous phthalates, likely to have large contributions from inhalation, are associated with subclinical airway inflammation in children.

## Introduction

Phthalates are a group of high-production volume compounds added to plastics to confer flexibility, and used in personal care and other consumer products, as well as in home materials such as vinyl flooring (Schettler 2006). Several phthalates are endocrine disrupting compounds in animal models (Howdeshell et al. 2008), and human epidemiologic studies have associated early life exposure to several phthalates with childhood eczema and asthma, as well as adverse neurobehavioral and reproductive effects (Bornehag and Nanberg 2010; Swan 2008; Swan et al. 2009; Whyatt et al. 2011). Numerous biomonitoring studies, including the U.S. National Health and Nutrition Examination Survey (NHANES), have monitored phthalate metabolites in urine and shown widespread exposure to many phthalates, including among children and inner-city populations (Adibi et al. 2008; CDC 2010; Kato et al. 2005; Silva et al. 2004; Teitelbaum et al. 2008). Although phthalates are rapidly metabolized, the ubiquity of these metabolites in urine suggests that exposure occurs nearly constantly with contributions from ingestion, inhalation, and dermal absorption (Frederiksen et al. 2007). Personal air concentrations of two phthalates, diethyl phthalate (DEP), which is more volatile than most other phthalates, and butylbenzyl phthalate (BBzP), which is also found on respirable particles, were correlated with maternal urinary metabolite concentrations (rho = 0.27 and rho = 0.48) (Adibi et al. 2008). Thus inhalation is one potentially important route of exposure for these two phthalates with dietary exposure more important for several other phthalates, including di-*n*-butyl phthalate (DnBP) and di(2-ethylhexyl) phthalate (DEHP) (Wormuth et al. 2006). Children have higher urinary concentrations than adults to many of the phthalate metabolites (CDC 2010; Silva et al. 2004; Wormuth et al. 2006), indicating the need for additional study of the potential health consequences of these exposures.

Fractional exhaled nitric oxide (FeNO) is a non-invasive biomarker of airway inflammation that is associated with eosinophilic inflammation (Dweik et al. 2011). FeNO is an important marker of subclinical airway inflammation predictive of future respiratory health among asthmatic and non-asthmatics. Prospective epidemiologic studies have associated higher FeNO with an increased risk of new-onset asthma in children and new-onset wheeze in adults (Bastain et al. 2011; Olin et al. 2010). FeNO also predicts exacerbations in asymptomatic asthmatic children after withdrawal of inhaled corticosteroids (Pijnenburg et al. 2005). Corticosteroid therapy but not bronchodilator usage reduces FeNO among children with acute asthma (Baraldi et al. 1997; Byrnes et al. 1997). FeNO is also responsive to environmental pollutants that contribute to respiratory health problems. Exposure to local air pollutants such as respirable particulate matter ($PM_{2.5}$) and black carbon have been associated positively with FeNO as has formaldehyde in the home (Cornell et al. accepted 2011; Delfino et al. 2006; Franklin et al. 2000; McCreanor et al. 2007).

In a cross-sectional case-control analysis of Swedish children ages 3-8, those with physician-confirmed asthma had higher bedroom dust concentrations of DEHP while higher bedroom dust concentrations of BBzP were found among those with physician confirmed rhinitis and eczema, allergic diseases that may be related to an asthmatic phenotype (Bornehag et al. 2004). In a cross-sectional analysis of *n*=240 adult NHANES participants with concurrent phthalate metabolite and lung function measures collected 1988-1994, urinary concentrations of the sum of mono-*n*-butyl phthalate (MnBP), a metabolite of DnBP, and mono-isobutyl phthalate, a metabolite of di-isobutyl phthalate, were associated with decreases in forced expiratory volume in 1 sec ($FEV_1$), forced vital capacity (FVC), and peak expiratory flow (PEF), but only among males (*n*=100). Monoethyl phthalate (MEP), the metabolite of DEP, was also associated with decreases

in FEV1 and FVC only among males, and both estimates were for decreases of greater than 100 ml per interquartile range change in the log-adjusted metabolite concentration (Hoppin et al. 2004).

Based on these prior human observational studies, we hypothesized that higher urinary concentrations of four phthalate metabolites: MEP, MnBP, monobenzyl phthalate (MBzP; the metabolite of BBzP), and mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP; a metabolite of DEHP) would be associated with higher concurrent FeNO in children. We also hypothesized that children with seroatopy and wheeze, as surrogate measures of allergy and the hyperresponsive airways of asthma, would be more susceptible to airway inflammation triggered by these environmental exposures.

**Methods**

Participants were selected based on available samples (requirements for adequate samples are discussed below) from the children in the Columbia Center for Children's Environmental Health (CCCEH) study, a longitudinal birth cohort that enrolled *n*=727 pregnant nonsmoking Dominican and African American women free of hypertension, diabetes, and known HIV living in Northern Manhattan and the South Bronx between 1998-2006 (Perera et al. 2003). Informed consent approved by the institutional review board of Columbia University and the Centers for Disease Control and Prevention was obtained from all participants. Children in the current analysis (ages 4.9-9.5 years) provided a spot urine sample collected by research staff during a home or office visit within 7 days of an FeNO measure that was collected in a clinic or office setting. Of the 280 children enrolled who provided at least one measure for both phthalates and FeNO, 89 had two measures, and two children had three, resulting in a total of *n*=373 paired observations of phthalate metabolites and FeNO.

Urinary phthalate metabolites were measured at the Centers for Disease Control and Prevention as previously described (Adibi et al. 2008; Kato et al. 2005). DEHP has several highly correlated metabolites in urine so we used the concentration of MEHHP as our proxy for exposure to DEHP because it has a longer half-life, higher average concentrations, and higher detection frequency than the monoester metabolite mono(2-ethylhexyl) phthalate (MEHP) (Preau et al. 2010). In this dataset, those two metabolites had a rank correlation of 0.85 ($p<0.001$, $n=373$).

Following guidelines from the American Thoracic Society (American Thoracic Society and European Respiratory Society 2005), FeNO was collected with a modified offline device (#CBSK 01400, GE Instruments, Boulder, CO) appropriate for collection of FeNO from children as briefly summarized in the appendix and described previously (Perzanowski et al. 2008; Perzanowski et al. 2010; Rosa et al. 2011). A gas syringe was used to collect samples of ambient nitric oxide (NO) at the time of collection. FeNO concentrations were quantified via chemiluminescence. FeNO data were excluded if the child had a cold at the time of the visit, did not inhale through the ambient NO scrubber, or if the room of the test had over 100 ppb ambient NO.

Seroatopy was defined as specific IgE to dust mite, cockroach, or mouse allergens ($\geq$0.35 IU/ml) by ImmunoCap (Phadia, Uppsala, Sweden), in the closest serum sample collected within one year of the FeNO measure (Donohue et al. 2008). This measure was available for 86% of observations ($n=322/373$) from 248 children. Because phlebotomy often coincided with the collection of other specimens and data, a sensitivity analysis was performed restricting to $n=167$ observations with seroatopy and FeNO measured in samples collected on the same day.

As a proxy measure for children who might have airway hyperresponsiveness, mothers were asked whether their child had wheezing or whistling in the chest in the past 12 months at age 5, 6, 7 and 9 years using the wheeze module of the validated International Study of Allergy and

Asthma in Children (ISAAC) questionnaire (Asher et al. 1995). A questionnaire was available from the day of the FeNO or the following 12 months for *n*=331 observations and a sensitivity analysis restricted the interaction analysis to the *n*=211 observations with wheeze questionnaires administered on the day of the FeNO measure. Because reports on single questionnaires may not characterize the episodic and variable timing of wheeze in childhood, we also used a larger set of repeated questionnaires to model the probability of wheezing in the last three months as a continuous measure at each FeNO collection for all *n*=373 observations. This probability was computed by multiplying each child's probability of belonging to four latent patterns (classes) of childhood wheeze by the probability of wheezing in the past three months at the age of collection for each pattern. Individual posterior probabilities of class membership and wheeze prediction equations were derived from a four-class latent class growth analysis (LCGA). This LCGA included 7048 questionnaires on report of wheeze in the past 3 months from 15 timepoints between 3 months and 9 years of age in a larger subset (*n*=689) of the children in the CCCEH study (Chen et al.).

*Statistical analysis*

Because individual children had one to three observations with phthalate and FeNO collections, repeated measures were modeled using generalized estimating equations (GEE), and confidence intervals and p-values associated with the regression coefficients were based on robust standard errors. All regression models included specific gravity as a covariate to adjust for urinary dilution (Barr et al. 2005), as well as a set of a priori potential confounders: age, sex, ethnicity, and ambient NO concentration. Concentrations of phthalate urinary metabolites, FeNO, and ambient NO were log transformed prior to analysis due to the right skew and high variance at the upper

range of their distributions. Regression parameter estimates are presented as percent differences in FeNO for a one log-unit higher urinary metabolite concentration. Analyses were conducted using R version 2.13.1 with the gee and ggplot2 packages for regression models and data visualization (Carey 2011; R Development Core Team 2011; Wickham 2009).

**Results**

The 280 participants included 144 girls and 136 boys, and were 66% Dominican and 34% African American. At the FeNO measure, 25% had a report of a smoker in the home, and participant ages ranged between ages 4.9 and 9.5 years (mean age 6.6, SD 1.6 years). Those included in the analysis did not differ from the remainder of the original cohort on major demographic factors collected at enrollment (see table E1 in Appendix). Exposures to the phthalates examined were widespread as suggested by the detection of the metabolites of all four phthalates in 100% of samples (*n*=373). Concentrations varied widely and the highest metabolite concentrations were for MEP as shown in Table 1. The concentrations of the four phthalate metabolites within each sample were positively correlated with a range of pair-wise Spearman's correlations from rho = 0.23 (MEP and MBzP) to rho = 0.69 (MnBP and MBzP).

**Table 1**. Distributional summary for unadjusted urinary metabolite concentrations (ng/ml) measured in 373 spot samples from 280 New York City children 4.9 to 9.5 years old.

| Metabolite | n | >LOD | Min | 25% | 50% | 75% | 95% | Max | GM (95%CI) |
|---|---|---|---|---|---|---|---|---|---|
| MEP | 373 | 100% | 11 | 83 | 169 | 347 | 1920 | 12100 | 182 (162 to 206) |
| MnBP | 373 | 100% | 3 | 24 | 48 | 85 | 212 | 539 | 44 (40 to 49) |
| MBzP | 373 | 100% | 1 | 15 | 32 | 70 | 354 | 2080 | 34 (30 to 39) |
| MEHHP | 373 | 100% | 2 | 20 | 46 | 87 | 385 | 1690 | 46 (41 to 52) |

To characterize predictors of children's urinary phthalate metabolite concentrations, GEE models with age, sex, and race/ethnicity as predictors were fit for each of the four log-transformed

metabolite concentrations also including specific gravity in the models as a covariate. The urinary concentrations of three of the metabolites were higher in females than in males: MEP (42% higher, 95% CI, 12% to 80%, p=0.004), MnBP (28% higher, 95% CI 7% to 53%, p=0.007), and MEHHP (34% higher, 95% CI 9% to 65%, p=0.005). Only the urinary concentration of MEHHP varied with age (increasing 10% per year, 95% CI 2% to 18%, p=0.02). There was no significant difference by race/ethnicity for the urinary concentrations of any of the metabolites.

*Phthalate urinary metabolites and FeNO*

FeNO measures ranged from 2.3 to 71.6 ppb with a median of 7.9 ppb and an interquartile range from 5.7 to 12.4 ppb and were consistent with a log-normal distribution. In a GEE model of FeNO with age, sex, race/ethnicity, and ambient NO concentration as predictors, both age and ambient NO concentration were positively associated with FeNO. In four separate adjusted models, FeNO was associated with urinary concentrations of MEP, MnBP, and MBzP but not MEHHP, after controlling for specific gravity, age, sex, race/ethnicity, and ambient NO concentration (Table 2). The effect size for MnBP was slightly larger than for MEP and MBzP although all three were very similar and were consistent with linearity in adjusted models using quintiles of urinary concentration (Figure 1). For example, there was a 7.6% increase in FeNO (95% CI 2.2% to 13.3%, p=0.005) for each log-unit increase in MBzP concentrations, that would correspond to an FeNO concentration of 9.1 versus 8.1 ppb for a child at the $75^{th}$ versus the $25^{th}$ percentile of MBzP concentrations, assuming mean levels of covariates. Similarly, a log-unit increase in MEP concentration was associated with a 7.0% increase in FeNO (95% CI 1.6% to 12.6%, p=0.008) adjusted for covariates, while a log unit change in MnBP concentration was associated with a 9.9% increase in FeNO (95% CI 1.8% to 18.8%, p=0.014) adjusting for

covariates. There was no significant interaction by child sex, and adjusting for having a smoker in the home did not change the magnitude or significance of estimates (data not shown). A simulation study was also used to explore the magnitude of confounding necessary to see the observed association (see Appendix). In a sensitivity analysis restricted to the subset of FeNO and urine samples from the same day (84% of observations, *n*=313), the estimates for MEP, MnBP, and MBzP were largely unchanged (beta estimates were 94%, 87%, and 90% of the beta estimates from the full dataset) and remained statistically significant, see Table E1 in the appendix.

**Table 2.** Adjusted[a] percent difference in fractional concentration of nitric oxide for a one log-unit higher urinary phthalate metabolite concentration from GEE regression models

| | Single metabolites, four separate models (*n*=373) | | |
|---|---|---|---|
| Metabolite | % Difference | (95% CI) | p-value |
| MEP | 7.0 | (1.6, 12.6) | 0.01 |
| MnBP | 9.9 | (1.8, 18.7) | 0.01 |
| MBzP | 7.6 | (2.2, 13.3) | 0.005 |
| MEHHP | 4.2 | (-1.5, 10.3) | 0.15 |
| | | | |
| | Single metabolites, separate models adjusted for seroatopy (*n*=322) | | |
| Metabolite | % Difference | (95% CI) | p-value |
| MEP | 6.7 | (1.2, 12.5) | 0.02 |
| MnBP | 9.3 | (1.0, 18.2) | 0.02 |
| MBzP | 8.4 | (2.8, 14.3) | 0.002 |
| MEHHP | 3.5 | (-2.7, 10.1) | 0.26 |
| | | | |
| | All four metabolites in one model adjusted for seroatopy (*n*=322) | | |
| Metabolite | % Difference | (95% CI) | p-value |
| MEP | 6.1 | (0.2, 12.4) | 0.04 |
| MnBP | -1.2 | (-10.7, 9.4) | 0.81 |
| MBzP | 8.2 | (1.7, 15.1) | 0.01 |
| MEHHP | 1.6 | (-4.5, 8.1) | 0.62 |

[a]All models adjusted for specific gravity, sex, race/ethnicity, age, and ambient NO concentration

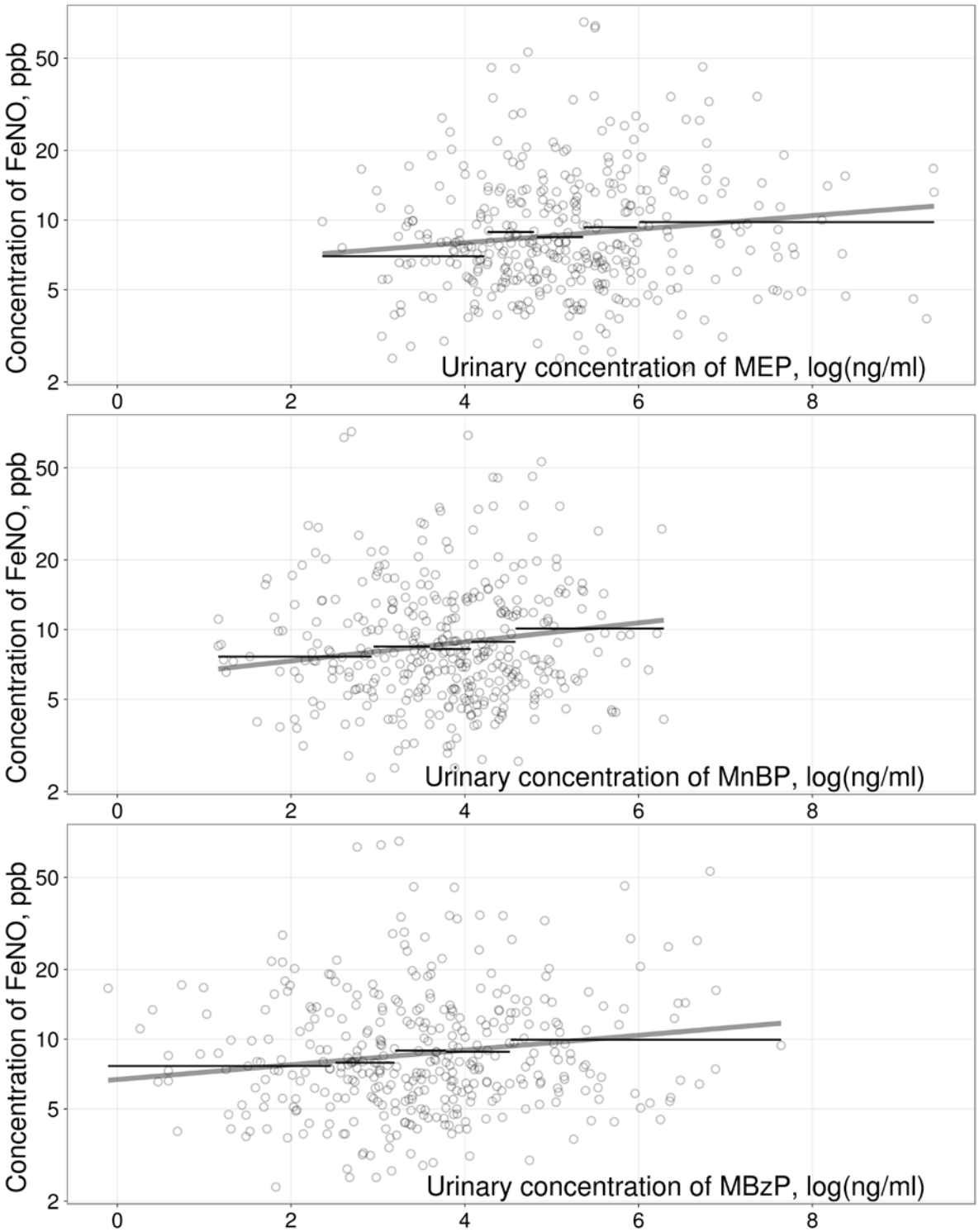


**Figure 1: The association between urinary concentrations of three phthalate metabolites: MEP, MnBP, and MBzP and FeNO from separate models (*n*=373 observations from 280 children).**

Adjusted estimates for each quintile are shown as horizontal segments. The continuous linear association is shown as a wider grey line. Both quintile and continuous model estimates are adjusted and shown at mean levels of specific gravity, age, sex, race/ethnicity, and ambient NO.

Using measures within one year, 32% were classified as seroatopic at their FeNO observation (*n*=104/322). Adjusting for seroatopy did not substantially alter the estimates for the individual phthalate metabolites (Table 2). In sensitivity analyses similar results were seen when restricting to seroatopy measured on the day of FeNO collection (*n*=167), see Table E1 in the Appendix.

In an adjusted multi-pollutant model of FeNO including concentrations of all four metabolites (MEP, MnBP, MBzP, and MEHHP) that was also adjusted for seroatopy, urinary concentrations of both MEP and MBzP remained statistically significant independent predictors of FeNO with similar effect size estimates to those in the single metabolite models as shown in Table 2. While MEHHP concentrations remained non-significant as in the single metabolite models, the single-metabolite beta estimate for MnBP decreased from 0.089 in the seroatopy adjusted model to a beta of -0.012 in the multi-pollutant model and was no longer statistically significantly associated with FeNO after adjusting for the other phthalate metabolite concentrations (p=0.81). Therefore, the MnBP association with FeNO seen in the single phthalate model may be due to the high correlation between MnBP and MBzP. In the adjusted four-pollutant model, a log-unit higher concentration of MEP and MBzP was associated with a 6.1% increase (95% CI 0.2% to 12.4%, p=0.04) and a 8.2% increase (95% CI 1.7% to 15.1%, p=0.01) in FeNO respectively after adjusting for all four metabolite concentrations, seroatopy, specific gravity, age, sex, race/ethnicity, and ambient NO.

*Interaction between phthalate metabolites and seroatopy or wheeze*

There was no direct association between the urinary concentrations of any of the four metabolites with incident seroatopy or reported wheeze (see Appendix).

As seen in Figure 2, FeNO was higher among those with seroatopy (geometric mean (GM) 11.6 ppb, *n*=104) than those without seroatopy (GM 7.4 ppb, *n*=218; p<0.001). Interaction terms found no significant difference in the slopes of the MEP or MBzP and FeNO associations between children with and without seroatopy (p=0.30 and p=0.43).

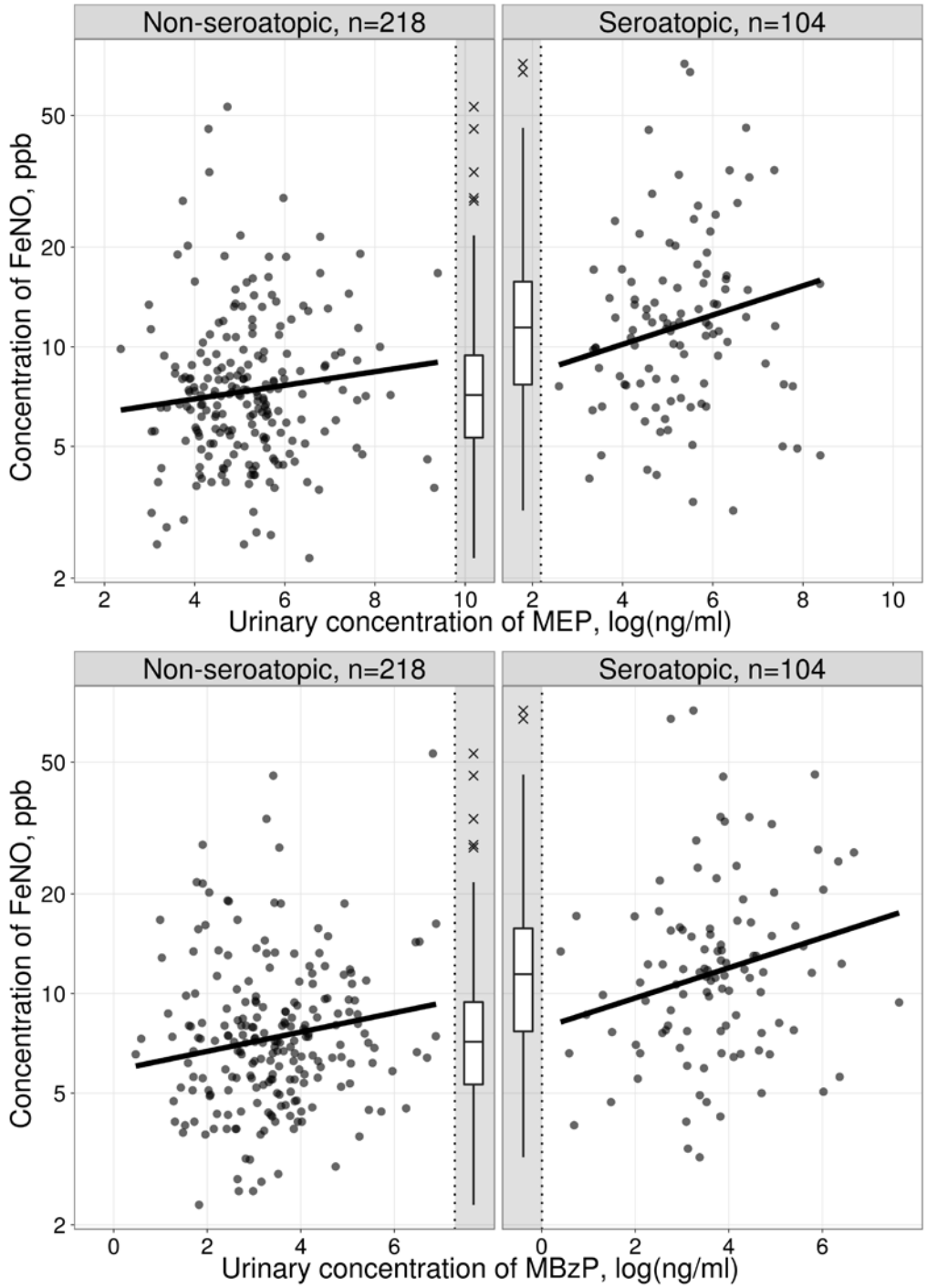


**Figure 2: The association between MEP or MBzP urinary concentrations and FeNO stratified by seroatopy of the child (*n*=218 and *n*=104 observations from 248 children).** Boxplots show the distribution of FeNO concentrations is higher among atopics than non-atopics ($p<0.001$). The interaction terms testing for a difference in slopes were not statistically significant ($p=0.30$ and $p=0.43$), shown at the mean specific gravity, age, sex, race/ethnicity, and ambient NO concentration. Main effects for both metabolites remained statistically significant after adjusting for seroatopy without interaction.

We also assessed the interaction between phthalate concentrations and wheeze using data from the ISAAC questionnaire responses to test whether children who wheeze are more susceptible to airway inflammation associated with phthalate exposure. Overall, 22% of children had a report of wheezing or whistling in the chest in the past 12 months at their FeNO observation or their next questionnaire collected within 12 months. There was no significant interaction between concentrations of MEP, MnBP, or MEHHP and report of wheeze in the past year in the association with FeNO. There was a significant positive interaction of the MBzP and FeNO association by ISAAC wheeze (Figure 3; p=0.038, *n*=331). In sensitivity analyses, this interaction was stronger and remained significant when restricted to ISAAC wheeze questionnaires asked on the same day as FeNO collection (p=0.009, *n*=211).

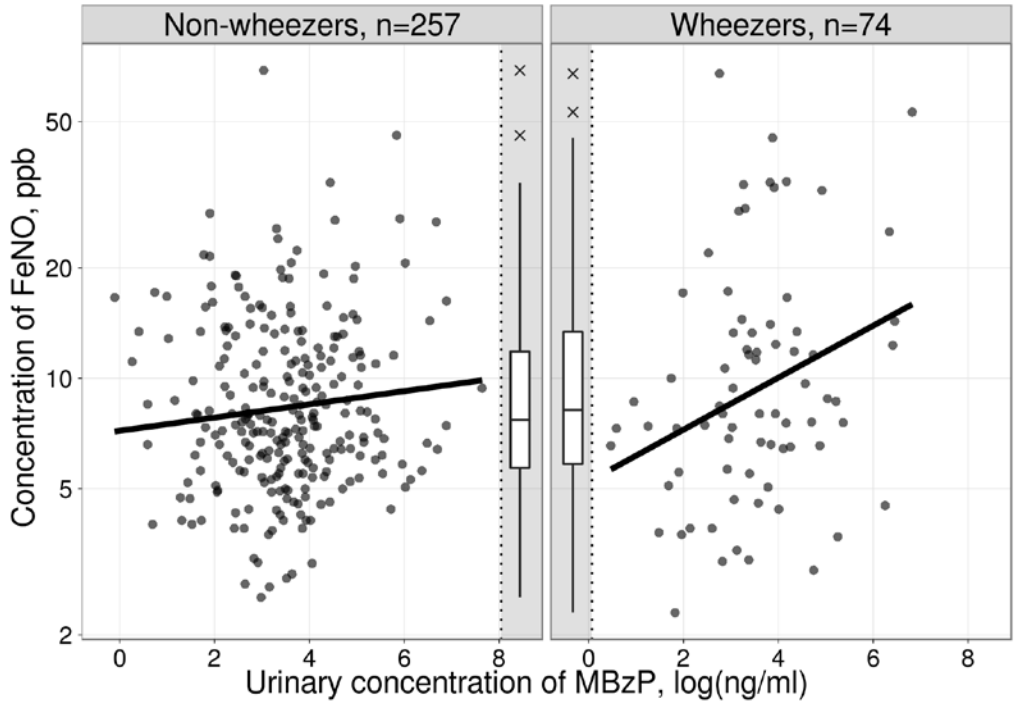


**Figure 3: The association between MBzP urinary concentrations and FeNO varies by whether the mother reported on the next questionnaire that the child had wheezing or whistling in the chest in the past 12 months (*n*=331 observations from 263 children).** The interaction term between MBzP and the report of wheeze was positive and significant (p=0.038) in the adjusted model shown at the mean levels of specific gravity, age, sex, race/ethnicity, and ambient NO.

As a further exploration of the interaction between phthalates and wheeze, a latent class growth analysis was used to model the probability of wheezing in the past three months. This continuous variable was available for all *n*=373 observations and probabilities ranged from 0.01 to 0.68 although they were highly skewed with a median of 0.04, a 75$^{th}$ percentile of 0.08, and a 95$^{th}$ percentile of 0.37. There was no evidence of interaction between concentrations of MEP, MnBP, or MEHHP and LCGA predicted probability of wheeze on FeNO. However, as in the analysis using a dichotomous ISAAC wheeze question, the interaction between urinary MBzP concentrations and the LCGA probability of wheezing on FeNO was positive and highly significant (p=0.004). The association between concentrations of MBzP and FeNO was of a larger magnitude among those with a higher probability of wheezing (Figure 4).

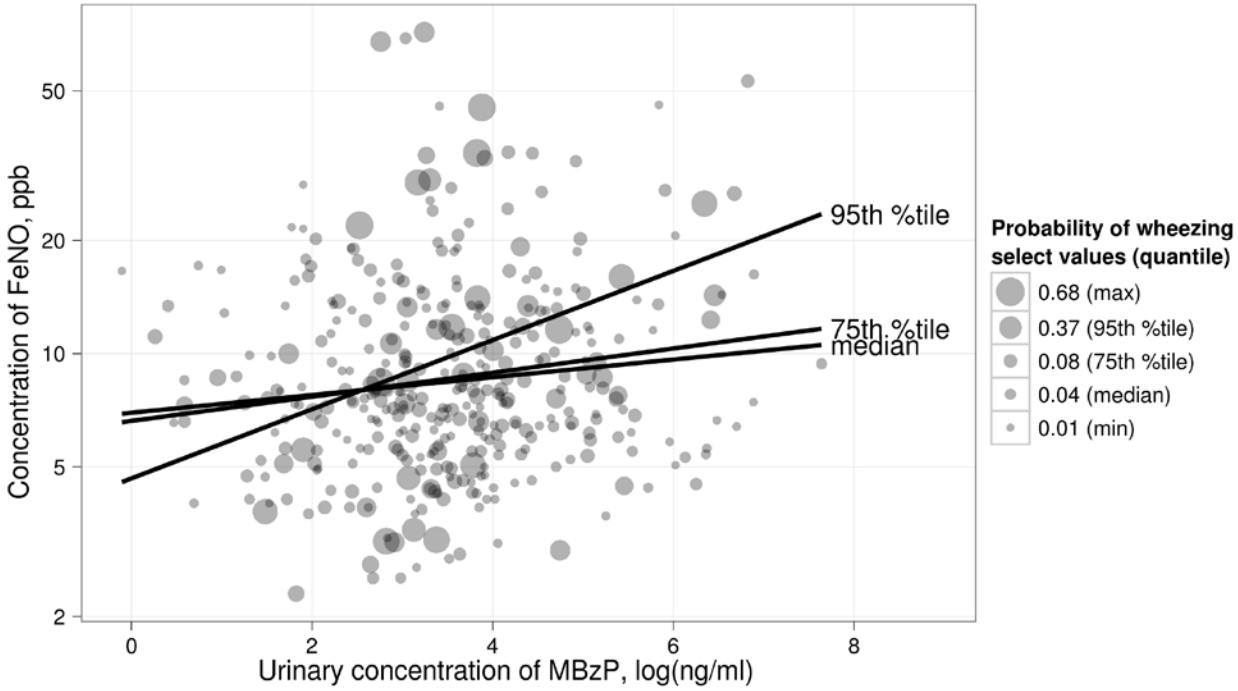


**Figure 4: The association between MBzP urinary concentrations and FeNO is stronger among children predicted to wheeze (*n*=373 observations from 280 children).**
The area of points is proportional to the probability of wheezing predicted from a latent class growth analysis (continuous variable ranging from 0.01 to 0.68). The three lines display the MBzP and FeNO association for participants at the median, 75$^{th}$ percentile, and the 95$^{th}$ percentile of the probability of wheeze shown at the mean specific gravity, age, sex, race/ethnicity, and ambient NO concentration from a multivariable GEE model. The interaction term between MBzP and the probability of wheezing was positive and highly significant in the model (p = 0.004).

**Discussion**

In this cross-sectional study, urinary concentrations of three phthalate metabolites (MEP, MnBP, and MBzP) were associated positively with FeNO in separate adjusted models. All three associations remained largely unchanged after adjusting for seroatopy. When all four metabolites (MEP, MnBP, MBzP, MEHHP) were put in the same adjusted model, MEP and MBzP remained independent predictors of higher FeNO, while concentrations of MnBP, which is highly correlated with MBzP, were no longer associated with FeNO. These results indicate that urinary biomarkers of exposure to two phthalates are associated with a measure of subclinical airway inflammation in children.

Seroatopy is an important predictor of FeNO (Rosa et al. 2011), and allergen exposure can explain one-third of the FeNO variability among atopics (Sordillo et al. 2011). There were no statistically significant interactions to indicate that the association between individual metabolite concentrations and FeNO varied by seroatopy, although our sample size may have limited our ability to detect interactions.

We hypothesized that asthmatic children with hyperresponsive airways, operationally defined based on report of wheeze, might be more susceptible to airway inflammation triggered by environmental exposures. Although this operational definition did not measure actual airway hyperresponsiveness and relied on subjective reports, we saw a positive interaction between MBzP urinary concentrations and wheeze when we dichotomously classified children based on the ISAAC question on wheeze in the past 12 months. There was also a positive interaction between urinary concentrations of MBzP and the probability of wheezing predicted from a latent class growth analysis of questionnaire data, indicating that the MBzP and FeNO association is stronger

among a subset of children who wheeze. Although wheeze itself is episodic, the LCGA-based prediction was based on the pattern of wheezing at up to 15 time points between 3 months and 9 years of age and may offer a more stable indication of children with respiratory health concerns than a single questionnaire response. An additional advantage of the LCGA-based wheeze prediction is that it could be computed for all observations and derived a continuous variable using a large number of questionnaires and therefore may also be more informative than a dichotomous classification. Direct comparison of our two wheezing measures is difficult however, because the LCGA derived probability of wheeze over 3 months was based on a different question than the ISAAC wheeze and we did not propagate uncertainty estimates from the original LCGA model into the LCGA-based probability of wheeze.

While the strength of the cross-sectional design is that it allows for the observation of effects that may be short-lived in response to exposures that vary over time, there are several important limitations. In an observational study, urinary concentrations of particular phthalate metabolites may also indicate exposure to other chemicals that share sources or chemical properties with the parent phthalates. For example, the mothers of the children in this analysis who reported the use of perfume or an index of uses of other personal care products during pregnancy had higher urinary concentrations of MEP than other pregnant women within the same cohort (Just et al. 2010), and can be expected to also have had higher exposure to other chemicals in those products, such as artificial fragrances as seen in sampling of indoor air (Rudel et al. 2010). The substantially higher urinary concentrations of MEP in girls than boys warrants further investigation as the burden of asthma and respiratory disease differentially increases for girls in adolescence, when exposure to DEP might be expected to remain high (Almqvist et al. 2008). Further, with phthalates, exposures occur to mixtures of correlated compounds. For example, in this study MnBP

and MBzP were highly correlated (rho = 0.69), which may be due to shared sources of exposure and the fact that MnBP is a minor product of BBzP (6%) (Anderson et al. 2001). MBzP has been shown previously to be a more reproducible biomarker than MnBP in spot urine samples collected over six months from children in New York City (Teitelbaum et al. 2008), its concentration may serve as a better marker of the mixture of related compounds than does the concentration of MnBP.

Urinary concentrations of MBzP have recently been shown to be associated with a non-specific marker of systemic inflammation in a large population-based analysis. MBzP concentrations were associated with increased serum C-reactive protein (CRP) in a dose-dependent fashion among 8346 NHANES participants (Ferguson et al. 2011). An interquartile range higher urinary MBzP concentration was associated with an average of 6.0% (95% CI 1.7% to 10.8%) higher CRP, and this association held in analyses restricted to younger NHANES participants 6-12 and 13-19 years old. However, the mechanism through which phthalates are associated with systemic or airway inflammation remains unclear. Some phthalate metabolites, especially MEHP and MBzP activate the ubiquitously expressed nuclear peroxisome proliferator-activated receptors (PPAR) α and γ (Hurst and Waxman 2003), which are ligand-activated transcription factors important in a variety of physiological processes including airway inflammation and airway remodeling. For example, it has been shown that the expression of PPARγ is higher in the bronchial submucosa, airway epithelium, and smooth muscle of asthmatics than controls and is associated with decrements in lung function (Benayoun et al. 2001).

Although there is an established and growing literature on the association between FeNO and components of local air pollution such as $PM_{2.5}$ and black carbon (Cornell et al. accepted 2011; Delfino et al. 2006), this observational study of children is the first reporting a positive association between biomarkers of exposure to phthalates and FeNO. These results contrast with those from

two small controlled chamber studies. In a study of 10 adult volunteers (5 asthmatics), there was no increase in post- versus pre-challenge FeNO, although there was an increase in respiratory symptoms, after 4 hours in an exposure chamber with 1 $m^2$ of PVC flooring shown to be releasing a breakdown product of DEHP (Tuomainen et al. 2006). In 18 healthy adult females, a non-significant increase in FeNO relative to pre-challenge was seen after 4.5 hr with 4 $m^2$ PVC surface stated as containing DEHP and BBzP, although FeNO decreased in the reference condition resulting in a significant difference from the challenge (Kolarik et al. 2009).

**Conclusions**

We report cross-sectional associations between children's urinary concentrations of three phthalate metabolites and FeNO, a marker of airway inflammation. Concentrations of both MEP and MBzP remained associated with FeNO in a four-pollutant model. The association of concentrations of MBzP and FeNO was significantly stronger among children with reported wheeze presumed to have hyper-reactive airways more susceptible to environmental exposures.

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**Disclaimer**

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the CDC.

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## Appendix 1

*Sample collection of FeNO*

The protocol for FeNO collection has been described previously (Perzanowski et al. 2008; Perzanowski et al. 2010). FeNO was collected in mylar bags after diverting to discard the first portion of the breath, approximately 1-3 seconds of exhalation of dead-space air, using an offline device (#CBSK 01400, GE Instruments, Boulder, CO). Children were coached through attempts to gather three valid maneuvers in which they exhaled at 83 ml/sec and had either inhaled through the NO scrubber or there was an ambient NO of less than 20 ppb in the testing room. Data analysis used the mean of the valid attempts for each child during their visit.

## References to Appendix

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**Appendix 2**

*Demographic characteristics and sensitivity analyses*

**Table E1.** Characteristics of mother-child pairs included and excluded from analysis

| Characteristic | Participants included[a] *n*=280 | Participants excluded[b] *n*=447 | p-value |
|---|---|---|---|
| Mother's Age: Mean (IQR)[c] | 25 (21, 29) | 25 (21, 29) | 0.74 |
| Mother's Ethnicity (%) | | | 0.83 |
| African American | 34 | 35 | |
| Dominican | 66 | 65 | |
| Mother completed high school, GED, or greater (%)[d] | 65 | 63 | 0.62 |
| Never Married (%)[d] | 69 | 64 | 0.17 |
| Maternal Asthma (%)[d] | 24 | 21 | 0.39 |
| Child's Sex (% male) | 49 | 48 | 0.96 |
| Child's Seroatopy (%)[e] | | | |
| at 60 months | 24 | 27 | 0.51 |
| at 108 months | 39 | 37 | 0.83 |
| Child's Wheeze (%)[f] | | | |
| at 60 months | 25 | 26 | 0.94 |
| at 108 months | 22 | 31 | 0.12 |

GED, General Educational Development; GM, geometric mean; IQR, interquartile range
[a]Missing: Education (*n*=1), Never Married (*n*=1), Child's seroatopy at 60 months (*n* =32), 108 months (*n*=157), Wheeze at 60 months (*n*=15), Wheeze at 108 months (*n*=95)
[b]Missing: Education (*n*=13), Never Married (*n*=5), Child's seroatopy at 60 months (*n*=208), 108 months (*n*=341), Wheeze at 60 months (*n*=220), Wheeze at 108 months (*n*=313)
[c]Age at delivery; assessed with Wilcoxon rank sum test
[d]Data from the prenatal questionnaire
[e]Seroatopy defined as ≥0.35 IU/ml specific IgE to cockroach, dust mite, or mouse
[f]Wheeze from ISAAC question on wheezing or whistling in the chest in the past 12 months

There were no significant differences between those included in the analysis (*n*=280) and the remainder of the original cohort (*n*=447) in univariate testing. Similarly, a multivariable logistic regression model built with prenatal variables (age, ethnicity, education, marital status, maternal asthma, child sex, *n*=710) found no significant difference between those included and excluded from analysis. The large rate of missing data in the measures of seroatopy and wheeze for excluded children at ages 5 and 9 years did not allow their inclusion in the multivariable model.

**Table E2:** Adjusted[a] percent difference in fractional concentration of nitric oxide for a one log-unit higher urinary phthalate metabolite concentration from sensitivity analyses of GEE regression models restricting to urine samples collected on the same day as FeNO or adjusted for a subset of seroatopy samples collected on the same day as FeNO.

| | Single metabolites measured same day, four separate models (*n*=313) | | | Single metabolites, four separate models adjusted for same day seroatopy (*n*=167) | | |
|---|---|---|---|---|---|---|
| Metabolite | % Difference | (95% CI) | p-value | % Difference | (95% CI) | p-value |
| MEP | 6.6 | (1.0, 12.5) | 0.02 | 8.3 | (1.0, 16.0) | 0.02 |
| MnBP | 8.6 | (0.1, 17.8) | 0.04 | 10.3 | (0.3, 21.3) | 0.04 |
| MBzP | 6.9 | (1.1, 13.0) | 0.02 | 6.9 | (-0.5, 14.9) | 0.06 |
| MEHHP | 2.6 | (-3.5, 9.1) | 0.39 | 3.5 | (-5.3, 13.2) | 0.44 |

[a]All models adjusted for specific gravity, sex, race/ethnicity, age, and ambient NO concentration

**Appendix 3**

*Simulation of confounding*

As a simulation exercise, we also used fake data to explore how strong a confounder would be needed to see an association such as we have reported if our analyzed exposure were only associated with the outcome through a correlated confounder such as another chemical component of personal care products. We explored a range of correlations between the confounder and the analyzed exposure as well as several values of the assigned association between the confounder and the outcome. To create a simple simulation that was comparable with our dataset, we generated repeated draws of n=373 independent samples for the correlated exposure variables from a random bivariate normal distribution with prespecified population level means and variances to match those of MEP concentrations from children in our study. The correlation between the confounder and analyzed exposure was varied from r = 0.10 to 0.60. In a recent study collecting 24-hour indoor air samples, the concentration of DEP had a Kendall's tau correlation with other common semivolatile endocrine disrupting compounds in indoor air in the range of 0.25 to 0.45 (Rudel et al. 2010). The strength of the association of the confounder with the outcome variable was set to be approximately equal to, two times, or four times the strength of the observed association from the single pollutant model in this study (beta = 0.07, 0.14, or 0.28). In generating the outcome variable, error was added such that the coefficient of determination for the association of the confounder and outcome was $R^2 = 0.15$, a value that is typical of molecular epidemiologic associations (sensitivity analysis showed similar patterns at $R^2 = 0.30$, data not shown). Univariate regression models were built using only the correlated exposure (rather than the confounder directly associated with the outcome), and the regression coefficients were stored and averaged

over 100 simulations for each of the 11 levels of the correlation and 3 levels of the confounder beta.

As expected, results showed that the apparent effect size of the analyzed exposure in the presence of a confounder increased with larger correlations between these exposures and was higher with larger effect sizes for the confounder. Simulation results show that in order to see an effect size of the magnitude observed for MEP in our data, a confounder would need to have either twice the effect size and a correlation of at least r = 0.5 or else have four times the effect size with a correlation of at least r = 0.25.

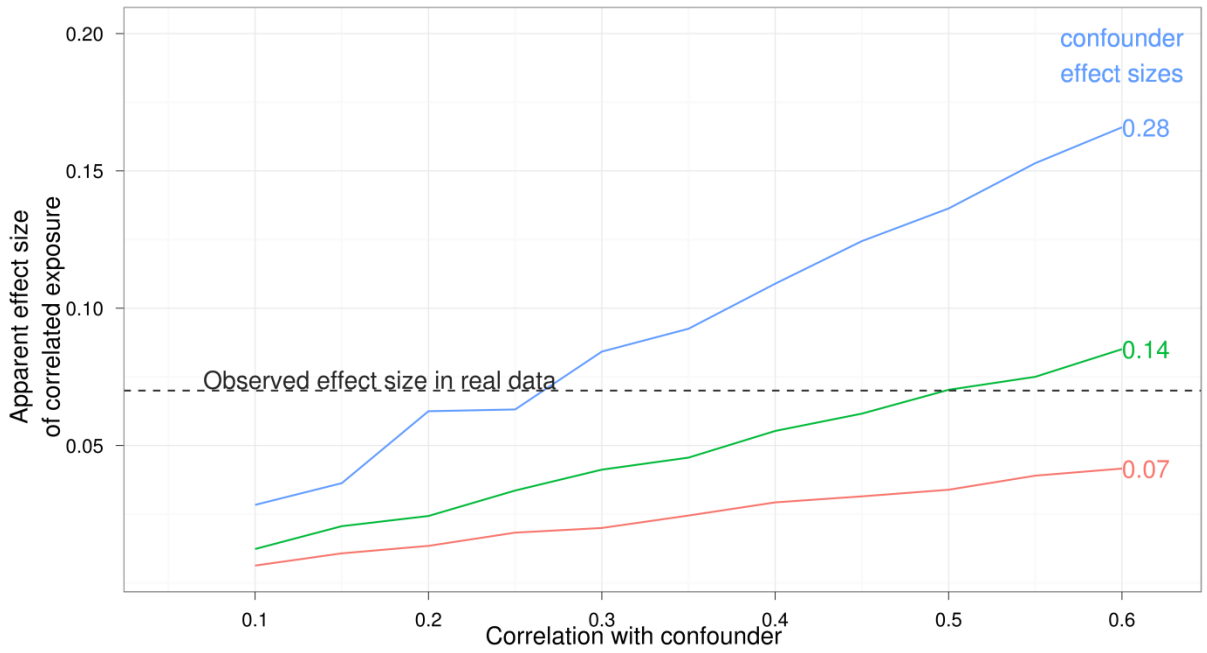


**Figure E1: Simulation of observed effect sizes under varying degrees of correlation with a confounder of differing strengths**

This simulation, while simplified to consider only a single exposure and single confounder, demonstrates that a correlated exposure acting as an unobserved confounder would need a considerably larger true effect size to induce the observed association between MEP and FeNO if there was no such direct association.

## Appendix 4

*Analysis of postnatal urinary phthalates and incident seroatopy*

While exposure to phthalates is likely to occur at all ages, a cross-sectional analysis with seroatopy may be inappropriate as the onset of allergic sensitization to aeroallergens is largely non-reversible and incidence is spread over the childhood period. However, repeated sampling of both urinary phthalate concentrations and seroatopy at various ages in this cohort makes possible an analysis of the association of childhood exposure with later incident seroatopy. This analysis can be done on a larger subset of children in the CCCEH study without restricting to those with measures of FeNO.

*Methods*

Urinary metabolite concentrations were measured in urine samples collected at age 3 and 5 years at the CDC as previously described. Seroatopy at a particular age was defined as specific IgE (≥0.35 IU/ml) to dust mite, cockroach, or mouse in samples from age 2 or 3 years (combined), age 5, or age 7. Incident seroatopy at age 5 or 7 years was defined as new cases of seroatopy among those who had one or more samples collected at previous ages and had no previous positive specific IgE results. Thus, a child developing seroatopy between age 3 and 5 would not be included in the susceptible population at age 7. Logistic regression models using generalized estimating equations modeled incident seroatopy at age 5 and 7 and phthalate metabolite urinary concentrations two years prior adjusting for specific gravity, sex, race/ethnicity, age at atopy measure, and time between the phthalate and atopy measure.

*Results*

There were 240 children with phthalates measured at age 3, no seroatopy at age 2 or 3, and a seroatopy measure at age 5, as well as 147 children with phthalate measured at age 5, no seroatopy in available samples from ages 2, 3, or 5, and a measure of seroatopy at age 7. Overall, there were 387 observations with these available data from 295 children. There were 41/240 (17%) and 24/147 (16%) new incident cases of seroatopy at age 5 and 7 years respectively. There was no association between any of the individual metabolites of interest and incident seroatopy in the following two years (Supplemental Materials Table E3).

**Supplemental Materials Table E3: Adjusted Odds Ratios for a log unit change in children's phthalate metabolite concentrations at age 3 or 5 and incident seroatopy over the following two years.**

| Metabolite | OR | 95% CI | p-value |
|---|---|---|---|
| MEP | 1.20 | (0.96 to 1.53) | 0.12 |
| MnBP | 0.89 | (0.63 to 1.24) | 0.47 |
| MBzP | 1.09 | (0.89 to 1.34) | 0.37 |
| MEHHP | 1.13 | (0.82 to 1.57) | 0.45 |

*Discussion*

We have reported previously that there was no association between a maternal urinary concentration of MBzP and the development of seroatopy by age 5 years in this population (*Just et al. Submitted. 2011*). In this analysis we also did not see any association between phthalate metabolite concentrations measured at age 3 or age 5 and the development of seroatopy over the following two years.

## Appendix 5

*Phthalate metabolites and wheeze*

In both the subset with FeNO data and a larger group of children from the full CCCEH cohort with available data, there was no consistent association between individual phthalate metabolite concentrations for the four metabolites of interest and concurrent report of wheeze at age 5 or at age 7 (Supplemental Materials Table E3). Although MEHHP appeared positively associated with wheeze at age 7, this attenuated and was no longer significant in the larger dataset.

**Supplemental Materials Table E4:** Adjusted[a] odds ratio estimates for report of wheeze in the past year on ISAAC questionnaires at age 5 and 7 for a log unit increase of urinary phthalate metabolite concentration from separate single metabolite models restricted to kids in the FeNO analysis and those with available data in the larger CCCEH dataset

| **Age 5 ISAAC Wheeze (*n*=232)** | | | |
|---|---|---|---|
| Metabolite | OR | (95% CI) | p-value |
| MEP | 0.82 | (0.62, 1.07) | 0.14 |
| MnBP | 0.89 | (0.63, 1.26) | 0.50 |
| MBzP | 0.92 | (0.72, 1.18) | 0.52 |
| MEHHP | 1.06 | (0.78, 1.45) | 0.70 |

| **Age 5 ISAAC Wheeze (*n*=348)** | | | |
|---|---|---|---|
| Metabolite | OR | (95% CI) | p-value |
| MEP | 0.91 | (0.74, 1.12) | 0.37 |
| MnBP | 0.92 | (0.71, 1.20) | 0.54 |
| MBzP | 1.00 | (0.82, 1.21) | 0.98 |
| MEHHP | 1.11 | (0.85, 1.46) | 0.42 |

| **Age 7 ISAAC Wheeze (*n*=175)** | | | |
|---|---|---|---|
| Metabolite | OR | (95% CI) | p-value |
| MEP | 1.05 | (0.77, 1.44) | 0.75 |
| MnBP | 1.12 | (0.71, 1.76) | 0.61 |
| MBzP | 1.11 | (0.81, 1.51) | 0.50 |
| MEHHP | 1.37 | (1.01, 1.87) | 0.04 |

| **Age 7 ISAAC Wheeze (*n*=289)** | | | |
|---|---|---|---|
| Metabolite | OR | (95% CI) | p-value |
| MEP | 1.09 | (0.86, 1.37) | 0.47 |
| MnBP | 0.91 | (0.67, 1.24) | 0.55 |
| MBzP | 1.07 | (0.85, 1.33) | 0.56 |
| MEHHP | 1.19 | (0.91, 1.54) | 0.19 |

[a]Models adjusted for specific gravity, sex, and race/ethnicity

Rudel RA, Dodson RE, Perovich LJ, Morello-Frosch R, Camann DE, Zuniga MM, et al. 2010. Semivolatile endocrine-disrupting compounds in paired indoor and outdoor air in two northern California communities. Environ Sci Technol 44(17): 6583-6590.

## Chapter 8: Conclusion and future directions

*Summary of major findings*

The studies in the previous five chapters were designed to address a single unifying hypothesis of this dissertation: **Early life exposure to a mixture of phthalates will have associations with adverse allergic and respiratory health outcomes in children.** The components of this hypothesis were addressed in three working hypotheses with specific aims that dealt with exposure, statistical methods, and associations with health outcomes:

*Hypothesis 1:*

We predict that the use of personal care products and flooring materials in the home environment contribute to patterns of prenatal and childhood phthalate exposure. Specifically, we hypothesize that the use of perfume and other personal care products is associated with higher exposure to DEP and DnBP and that the presence of vinyl flooring materials that may contain phthalates is associated with higher exposure to BBzP but not DEHP.

*Principle findings from Specific Aim 1:*

Consistent with our hypothesis, the use of perfume and an index of other personal care products was associated with higher urinary concentrations of MEP among pregnant women. Contrary to our hypothesis, there was no association between use of nail products and MnBP. The lack of correlation between personal air DEP and urinary MEP concentrations among perfume users suggests that DEP exposure subsequent to perfume use, which is often applied directly to skin, may have a substantial dermal component not sampled by personal air monitoring. The correlation of air and urinary concentrations among non-users of perfume indicates that inhalation is an important potential route for exposure from other sources of DEP. Consistent with our

hypothesis, homes with flooring categorized as “vinyl or linoleum” had higher concentrations of BBzP in their indoor air, although, contrary to our hypothesis, they did not have higher concentrations of indoor air DEHP. Further, children living in homes with “vinyl or linoleum” flooring had higher urinary concentrations of MBzP. As anticipated, we found no correlation between indoor air DEHP and urinary DEHP metabolite concentrations. We did not see any association between flooring type and DEHP metabolite concentrations. Taken together, these two exposure studies establish that routine behaviors and residential materials may make a substantial contribution to exposure to DEP and BBzP in pregnant women and children.

*Hypothesis 2:*

We hypothesize that simple Bayesian regression models can estimate associations between multiple correlated phthalate biomarker measures and a continuous health outcome and that these estimates will better reflect actual mixtures and be more stable than estimates from single biomarker or full linear regression models.

*Principle findings from Specific Aim 2:*

We constructed two simple Bayesian regression models with resulting effect size estimates for individual phthalate metabolites that were more realistic than those from models that consider each metabolite separately. Further, they were more stable with smaller variances than from a full regression model. The flexibility of a Bayesian model that groups similar predictors was particularly helpful as it may be inappropriate to assume similar parameter estimates for other covariates, including potential confounders. While no single metabolite predominated the association with reduced gestational age, the three most negative effect estimates were for mono(3-carboxypropyl) phthalate, a metabolite of high molecular weight phthalates including di-*n*-octyl

phthalate and two DEHP metabolites, mono(2-ethylhexyl) phthalate and mono(2-ethyl-5-hydroxyhexyl) phthalate.

*Hypothesis 3:*

We predict that higher prenatal exposure to butylbenzyl phthalate will increase risk of early childhood eczema and production of proallergic immunoglobulin E (IgE) antibodies by age five years. We also predict that exposure to the four phthalates will be associated with increased concurrent airway inflammation (FeNO) among cohort children.

*Principle findings from Specific Aim 3:*

We found that prenatal urinary concentrations of MBzP were associated with report of child eczema. Associations were strongest for eczema that was reported by child age 24 months. There was no association between prenatal MBzP concentrations and the development of seroatopy measured with total or specific IgE to indoor aeroallergens up to child age 60 months. Children's concentrations of MEP and MBzP were both associated with higher FeNO measured within seven days of the urine sample collection in a model that also included MnBP, MEHHP, and potential confounders. These associations did not appear to vary by seroatopy. However, the association between MBzP and FeNO was significantly stronger among children with wheeze.

*Connections between findings and broader relevance*

In our reanalysis of the association of eight phthalate metabolites with reduced gestational age (Chapter 5), we recommend Bayesian models as an alternative to conventional statistical approaches. In modeling children's urinary concentrations of MEP, MnBP, MBzP and MEHHP (Chapter 7), we report results from models fit to one metabolite at a time as well as a full model

with all four in as predictors. Consistent with our warnings from Chapter 5, we saw that the correlation of MnBP and MBzP resulted in the former having a significant effect that was completely null when all four metabolites were in the model. However, the impact of multicollinearity on estimate stability was much larger in the application of phthalate metabolites and reduced gestational age. Because the DEHP metabolites were associated with reduced gestational age, this subset of the most highly correlated metabolites needed to be considered carefully. However, in the absence of any association between MEHHP and FeNO, it was unnecessary in Chapter 7 to model additional DEHP metabolites that had rank correlations with MEHHP of $\geq 0.85$. As a result, our full model of four metabolites and FeNO in Chapter 7 did not exhibit the same instability as the full model with three DEHP metabolites and five other metabolites and gestational age in Chapter 5. Future work is needed to determine what degree of multicollinearity such full models can sustain before the resulting instability makes it necessary to utilize additional techniques, such as the Bayesian models in Chapter 5.

We found that personal care product use among the mothers was associated with higher MEP concentrations and that higher urinary concentrations of MEP in the children were associated with higher FeNO, a marker of airway inflammation. Our results suggest that personal care product use may be a modifiable source of exposure to DEP. However, interpretation of the association with airway inflammation is difficult because DEP, the parent phthalate of MEP, may be a proxy in these studies for a mixture of other semi-volatile or volatile organic compounds used in personal care products or other fragranced consumer products (e.g. air fresheners) (Peters 2005; Rudel et al. 2010; Steinemann 2009; Steinemann et al. 2011). While the association between volatile organic compounds and asthma or airway inflammation is complicated due to the large number of potential exposures, proxy measures have shown relevant associations (Mendell 2007; Nielsen et al. 2007).

For example, the children of women in the highest decile of reported use of chemical household products versus the lowest decile were more likely to wheeze persistently (OR 2.3, 95% CI 1.2 to 4.4) in a large European cohort study (Sherriff et al. 2005).

Two of our strongest and most consistent findings were related to urinary concentrations of MBzP, a biomarker that we have previously reported is the most reliable of the phthalate metabolites during pregnancy (Adibi et al. 2008). We found that prenatal urinary concentrations of MBzP were associated with early eczema, and that urinary concentrations in children were associated positively with airway inflammation. We also found that residential flooring materials were associated with exposure to BBzP, but not DEHP. In light of this finding on exposure, it is perhaps worthwhile reconsidering previous literature that has seen associations between vinyl flooring materials and asthma or bronchial obstruction (Bornehag et al. 2004; Jaakkola et al. 1999; Larsson et al. 2010; Mendell 2007; Oie et al. 1997). Although these associations have been presumed to be related to exposure to DEHP, our work in exposure as well as the associations with eczema and airway inflammation suggest that perhaps BBzP plays a key role in the exposure-response relation attributed to vinyl home materials.

**Future directions**

The studies in this dissertation have utilized environmental and biological samples to characterize exposure to phthalates, analyze effects of mixtures, and assess the association between prenatal and early-life exposure to four phthalates and risk of child eczema and airway inflammation. Future research will bridge across these areas of exposure science, statistical methodology, and health outcomes to better understand the role of phthalates in pediatric allergy and asthma.

*Identifying sources of phthalate exposure*

Exposure science plays an important role in source identification as well as exposure characterization for epidemiologic studies. In the previous chapters, questionnaires and visual inspection methods were used to gather information on potential sources of exposure and these were related to concentrations measured in environmental and biomonitoring samples. The methods used here appeared sensitive in classifying participants with the highest exposure, but lacked specificity because not all products identified as potentially containing phthalates likely did so. One recommendation to increase the specificity of characterizing exposed populations would be to use X-ray fluorescence (XRF) methods on materials in the home. XRF can detect chlorine and therefore differentiate polyvinyl chloride (PVC) materials, which often have phthalate plasticizers added to give them flexibility, from other types of plastic or non-plastic materials (e.g. linoleum). Cohort studies that conduct home visits could utilize portable XRF devices to improve upon visual inspection in the characterization of materials that may contain phthalates. This non-destructive additional screening might increase the specificity of exposure characterization by identifying flooring likely to contain phthalates without misclassifying linoleum or other surfaces as vinyl and without requiring a costly direct measurement of the flooring for phthalates. A more accurate characterization of flooring materials as PVC versus non-PVC, if validated by environmental sampling of phthalates, might be a useful proxy for chronic exposure to BBzP for future epidemiologic studies, although this would be of limited utility for retrospective exposure assessment among individuals without a stable residential history.

*Improving epidemiologic models with exposure information*

Controlled dosing studies have demonstrated that phthalates have urinary elimination half-lives of just a few hours (Anderson et al. 2001; Anderson et al. 2011; Koch et al. 2004; Schmid and Schlatter 1985). In two studies dosing adults, the modest relative standard deviation for phthalate metabolite concentrations between individuals of 20%-40% suggests that differences in metabolism can only explain a small portion in the biomarker variability seen in population studies (Anderson et al. 2001; Anderson et al. 2011). Instead, the concentrations of phthalate metabolites reflect the variability in exposure that can occur over short periods of time. We utilized this variability in these studies by examining cross-sectional associations of urinary metabolite concentrations with exhaled nitric oxide, a marker of airway inflammation that also varies over short temporal spans. For epidemiologic studies of more stable outcomes, the variability of the urinary phthalate metabolites, even over a single day, may be a challenge to characterizing exposure over a longer etiologic period. However, time-varying predictors of exposure can be used to correct for some sources of variability. For example, in a study that measured urinary metabolites in every urine void from eight adults over one week, there was a marked daily periodicity of the concentration of MEP, particularly on weekdays, that likely follows from the use of personal care products in the morning (Preau et al. 2010). If only single spot urines were available, combining information on time of day of collection with the measured urinary concentration would help to adjust where participant concentrations were relative to an expected daily average and might produce a more appropriate ranking of the inter-individual differences in exposure. In the context of a reproducibility study, the inclusion of time-varying predictors of exposure (such as time of day for MEP or time since last meal for DEHP since diet is the dominant route of exposure) would be expected to increase the intraclass correlation coefficient. This is

because reducing the intra-individual variability would increase the proportion of total variance that is between-persons.

Combining an understanding of exposure sources with data on biomarker variability may increase the reliability of biomarkers as dosimeters of average prenatal and postnatal exposure levels. Improving exposure characterization from biomarkers will be of particular use in epidemiologic studies of health outcomes with etiologic periods that span months to years such as asthma and allergy. Future studies that utilize methods for reducing measurement error such as two-stage models could reduce bias and increase power to detect associations between the unobservable average internal exposure to phthalates and health outcomes (Thomas et al. 1993). One way to implement these approaches would be to use substudies with more intensive sampling to construct a representative average exposure in a random subset of the participant population. These averages could be used as an outcome in models using single spot measures of urinary metabolite concentrations, specific gravity, time of collection, and information on major sources for each of the phthalates of interest. The parameters from these exposure models could be used to predict average exposure for the entire population which would then be used as a predictor in the second stage association with health outcomes. Structural approaches that do not directly estimate the latent parameter of internal or average exposure in the two-stage approach could also be implemented as a future direction but require more sophisticated statistical methods.

*Future studies of eczema and airway inflammation*

Future studies seeking to replicate the association seen here between early-life exposure to butylbenzyl phthalate and early childhood eczema should consider alternatives to maternal report of child's eczema to increase the objectivity of the assessment. For example, the standardized

SCORing Atopic Dermatitis (SCORAD) index, designed as a tool for clinical assessment and validated for interrater reliability, combines clinical skin examinations on extent and intensity of eczema with more subjective severity reports from the patient. This widely used tool could be collected during early clinical assessments to increase the objectivity of assessments and would likely increase the generalizability of study results as well.

We hypothesized that children who wheeze might have hyperresponsive airways and thus might be more susceptible to airway inflammation resulting from exposure to phthalates. We found that the association between MBzP and FeNO is stronger among children with report of wheeze than among children without wheeze in the past 12 months (Chapter 7). The interaction was even more significant when we used a prediction of wheeze resulting from the pattern of report of wheeze in the past three months on up to 15 questionnaires collected through childhood. However, this finding might be improved with a more refined operational definition of airway hyperresponsiveness. Rather than using report of wheeze on questionnaires, an operational definition for hyperresponsiveness could combine participant history with objective measures such as improvement in forced expiratory volume in one second ($FEV_1$) after bronchodilator as a measure of reversible airway obstruction. Clinical practice guidelines suggests that FeNO may be a particular useful marker of inflammation (e.g. as a result of persistent allergen exposure) or control (e.g. subsequent to inhaled corticosteroid therapy) among patients with eosinophilic asthma (Dweik et al. 2011). Thus, a more rigorous test of the hypothesis that the inflammatory response to phthalates is heightened among the susceptible could examine phthalate urinary metabolites and eosinophil counts from induced sputum samples from asthmatic and non-asthmatic children.

The combined results of specific aims 1 and 3 identified potentially modifiable sources of exposure to two of the phthalates, DEP and BBzP, which were subsequently associated with

airway inflammation in children. One potentially powerful study design to better understand these results would be to conduct exposure reduction interventions among children. Such a trial could use biomarker measures to confirm that exposures to these phthalates were reduced by the intervention (Rudel et al. 2011), and use measurements of FeNO before, during, and after the intervention to see if short-term exposure reductions led to concomitant changes in airway inflammation. Although evidence-based exposure reduction strategies have not been tested for these two phthalates, we would hypothesize based on our results that eliminating the use of fragranced products would substantially reduce exposure to DEP. Similarly, we would expect that relocating children to homes that do not contain vinyl flooring materials might lead to substantially lower exposure to BBzP. For example, epidemiologic studies could be timed so as to take advantage of changes in indoor environment that result from household moves, renovations, or changes in school location. Combining the results from these studies on exposure sources and epidemiologic associations for these phthalates indicates future areas for investigation in the respiratory health effects of exposure to phthalates.

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